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The interplay between inbreeding, infidelity and social environment in a cooperatively-breeding bird

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Author's Declaration

The work described in this thesis has been carried out by myself with guidance from my supervisors, Loeske Kruuk[1,2], Andrew Cockburn[2] & Craig Walling[1] , unless otherwise stated. The thesis is of my own composition and has not been submitted for any other degree or professional qualification.

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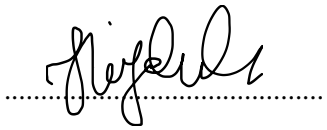

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An understanding of the natural world and what's in it
is a source of not only a great curiosity
but great fulfillment

– Sir David Attenborough

LAY SUMMARY

Mating patterns play an important role in the dynamics of natural populations. The causes, magnitude and consequences of infidelity and inbreeding - mating with relatives - are core topics in evolutionary and conservation biology. Interest in inbreeding stems from the very commonly observed reduction in an inbred individual's ability to survive and reproduce (fitness), termed inbreeding depression. Due to the ubiquity of inbreeding depression and given its negative consequences, it is often assumed that inbreeding should be avoided. Various mechanisms have been proposed as means of avoiding inbreeding, one of which is infidelity, where individuals mate outside of their social pair. However, theoretical work shows that inbreeding may also be advantageous, as it provides inclusive fitness benefits, through allowing an individual to increase the reproductive success of a relative. Therefore, determination of the extent to which individuals should avoid or prefer inbreeding is not straightforward. Moreover, it is unclear when (or whether) inbreeding avoidance can serve as an explanation for infidelity.

In this thesis I combine behavioural data I collected during fieldwork with detailed long-term records from a study of a superb fairy-wren (*Malurus cyaneus*) population based in the Australian National Botanic Gardens (Canberra, Australia). This is a wild and unmanaged bird population, presenting a rare opportunity to carry out complex analyses - including quantitative genetics modelling - on individuals living under natural conditions.

I use these data to investigate context-dependent mate choice, focusing on inbreeding and infidelity dynamics. Superb fairy-wrens have exceptionally high rates of infidelity, with the majority of offspring being sired by males outside of the social group. They are also cooperative breeders, where breeding pairs may be assisted by male helpers, which tend to be sons from previous broods. Therefore, they commonly live in groups formed of, and interact with, relatives. This added an extra dimension to my analyses, as I could explore the influence of social environment on both infidelity and inbreeding.

Starting with inbreeding and inbreeding depression, I have shown that inbreeding events were relatively rare, with only 6% of individuals being inbred. For inbreeding depression, I explored two traits: mass and survival. Heavier individuals had higher probability of survival, however I found inbreeding depression in mass, but not in survival. Upon further investigation I demonstrated that the association between mass and survival observed at a phenotypic level had no genetic basis and was instead caused by temporal factors. Moreover, social environment - the number of helpers in a group - did not affect inbreeding or inbreeding depression.

Although in a small proportion of cases, mothers may be socially paired to their sons, they never produced offspring with their sons: infidelity was 100% in the case of mother-son pairings. Further, observations of courtship behaviour I carried out during fieldwork showed that sons living in other social groups rarely visited and never displayed to their mothers. These results indicate that inbreeding avoidance of nuclear family inbreeding is driven by both females and males. However, beyond the cases of mother-son pairs, kinship between social partners did not predict infidelity. Therefore, overall it seemed unlikely that the high levels of infidelity in this population could be explained by inbreeding avoidance. Yet, extra-pair offspring were less likely to be inbred than within-pair offspring, suggesting that the spatial structure of the population may affect the probability of mating with relatives. Next, I explored the role of social environment in infidelity further.

The effects of social environment on infidelity were complex. First, I investigated the influence of the number of helpers: females assisted by more helpers had higher infidelity rates. In my consequent analysis, I showed that relatedness of helpers played a crucial role. Infidelity was higher in the presence of helpers unrelated to the breeding female, while the presence of helper-sons did not affect infidelity. These results excluded yet another explanation for the infidelity rates in the population - the 'constrained female' hypothesis.

In conclusion, I have shown that inbreeding and infidelity affect each other, but that the social environment has more influence on infidelity than on inbreeding. My results indicate that spatiotemporal factors play a major role in the dynamics of this superb fairy-wren population and suggest promising avenues for future research.

ABSTRACT

Despite a long-standing interest in understanding inbreeding, infidelity and cooperation in animal populations we still do not have a full picture of the interplay between these factors, especially in wild populations. However, the development of paternity assignment methods and statistical tools provides us with an opportunity to gain new levels of insight. In this thesis I combine behavioural data I collected during fieldwork with long-term records from a study of a superb fairy-wren (*Malurus cyaneus*) population based in the Australian National Botanic Gardens. I investigate mating strategies in general, and inbreeding avoidance in particular, as well as assess the role of social environment in context-dependent mate choice in this cooperative breeder.

First, in Chapter 2, I reconstruct a genetically-informed pedigree of the superb fairy-wren population using 26 years of data, in order to quantify the rates of inbreeding (only 6% of individuals were inbred) and extra-pair reproduction (61% of offspring had extra-pair paternity). I then test whether these high rates of infidelity might have evolved as a plausible mechanism for inbreeding avoidance in this population. I found evidence of inbreeding depression in nestling mass, but not in fledgling survival. Kinship between social partners did not predict infidelity, except in the case of mother-son social pairings, for which there was 100% infidelity. Nevertheless, extra-pair offspring were less likely to be inbred than within-pair offspring. Moreover, social environment - the number of helpers in a group - did not affect inbreeding or inbreeding depression. I concluded that, overall, inbreeding avoidance is unlikely to explain the extreme levels of infidelity in this study system.

In Chapter 3, I explore the effects of the social environment, and in particular different levels of relatedness of helpers in a group, on the mating patterns of the dominant female. My analysis demonstrated that whilst the presence of helper-sons did not affect a female's infidelity to her social partner, her rates of infidelity increased in the presence of unrelated helpers. The presence of unrelated helpers in a group was associated with increased rates of both extra-pair paternity and, unexpectedly, of extra-group paternity. Across a total of 1936 broods over 26 years, broods produced in groups assisted by at least one unrelated helper contained 67% extra-group offspring, compared to 58% in groups with only helper-sons. These findings suggest not only that the 'constrained female hypothesis' cannot explain the high levels of infidelity observed in this species, but also that the social environment can affect conspecific interactions in complex ways.

I then investigate mate choice and inbreeding avoidance from the perspective of the males in Chapter 4. Over two field seasons I collected behavioural data on male visits to female territories and data on courtship displays. I found that sons rarely visited ($<2\%$ of visits) and never displayed to their mothers. My results thus indicate that mother-son inbreeding avoidance is not driven entirely by females: males may face opportunity costs too and thus opt to exercise a level of mate choice through exercising strategic courtship.

Finally, in Chapter 5, I explore the factors driving the apparent selection on body size through juvenile survival identified in Chapter 2, by conducting a quantitative genetics analysis on nestling mass and fledging survival using mixed effects animal models. This approach allowed me to partition the variance in the two traits, and the covariance between them, into different sources, including additive genetic variance and covariance. I found that roughly two-thirds of the overall phenotypic variance in mass, and a similar proportion of the overall phenotypic variance in survival, were explained by the effects of nest, hatch date, cohort and additive genetic effect. Nest explained the largest proportion of variance in each of the traits: 43% in mass and 58% in survival. I demonstrated, through estimation of genetic covariances, that the association between mass and survival observed at the phenotypic level had no genetic basis and was instead caused by temporal factors (hatch date and cohort): the result indicates that there is no causal effect of mass on survival, and hence no potential for an adaptive response to selection despite heritability of both mass and survival.

I conclude with a discussion of the overall insights provided into the mating system and selection pressures in this wild cooperatively breeding songbird. I draw attention to the limitations of my analyses and suggest avenues for future research.

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Chapter 1

Introduction

1.1. Background Overview

An individual's reproductive success will be shaped by its interactions with conspecifics, the most important of which will be its choice of mate. Understanding the selection pressures that determine mate choice in different populations is crucial to explaining the diversity of mating systems we see across different animal populations. This thesis describes a study of mate choice and reproductive decisions in a wild bird population, using data from an exceptionally-detailed long-term study. I focus in particular on three important characteristics which still present major challenges for our understanding of mating systems: the occurrence or avoidance of inbreeding, the prevalence of infidelity and the existence of cooperative breeding. My study species, the superb fairy-wren (*Malurus cyaneus*), is a cooperative breeder known for exceptionally high levels of extra-pair paternity, but rates of inbreeding have not yet been analysed.

In this introductory section, I first provide a background to the study of inbreeding, inbreeding depression and inbreeding avoidance, then a brief overview of extra-pair mating (infidelity), and highlight the importance of studies of wild populations. I then outline cooperative breeding and the role of the social environment. I end the Introduction with a summary of information on the life history and ecology of superb fairy-wrens, and give a thesis overview.

Chapter 2 contains an analysis of rates of inbreeding and inbreeding depression, which allows me to test the extent to which its avoidance may be driving infidelity. In **Chapter 3**, I consider the interaction between cooperative breeding and extra-pair reproduction, which shows subtle effects of social environment on mate choice, and in **Chapter 4**, I explore levels of inbreeding avoidance further. Finally, in **Chapter 5**, I explore the role of cooperative breeding and the social environment in shaping selection pressures, by providing a fine-scale dissection of selection on juvenile development, and I close the thesis with a general discussion and conclusions in **Chapter 6**.

1.2. Inbreeding

1.2.1. Inbreeding and inbreeding depression

Mating between relatives is referred to as 'inbreeding', while mating between unrelated (or distantly related) individuals is termed 'outbreeding'. Related individuals are more genetically similar than unrelated individuals because they share a higher proportion of homologous alleles identical by descent. Therefore, when related individuals mate, they are more likely to pass on to their offspring copies of the same genes [Crow and Kimura, 1970], i.e. offspring of inbred matings are more likely to be homozygous at loci across the genome than are offspring of outbred matings. As a result, inbreeding may result in the reduction of fitness of the produced offspring - 'inbreeding depression' [Lynch and Walsh, 1998]. Two mechanisms are proposed as explanations for inbreeding depression: the existence of deleterious recessives (the 'dominance

hypothesis'), and the occurrence of overdominance [Lynch and Walsh, 1998; Roff, 2002; Carr and Dudash, 2003; Kristensen *et al.*, 2005]. The dominance hypothesis states that there is an increased probability of deleterious recessive alleles being expressed in inbred homozygous offspring. The reduction in fitness under this hypothesis therefore happens through the increase in the frequency of recessive homozygotes [Charlesworth and Willis, 2009]. On the other hand, the overdominance hypothesis focuses on heterozygote vigour - a situation where heterozygotes have higher fitness than homozygotes - and proposes that inbreeding leads to reduction in fitness through the decrease in the number of heterozygotes (loss of advantageous gene combinations) [Lynch and Walsh, 1998; Charlesworth and Charlesworth, 1999; Keller and Waller, 2002]. Under both scenarios, the average fitness of homozygotes is lower than that of heterozygotes, so the reduction in frequency of heterozygotes constitutes a loss of fitness. Identifying the mechanisms of inbreeding depression is possible, but requires experiments that are not feasible in most wild systems.

The existence of inbreeding depression will have multiple implications for many aspects of biological systems, such as the evolution of mating systems [Charlesworth and Charlesworth, 1987] and dispersal [Pusey, 1987], as well as population viability and persistence [Frankham, 1995; Wright *et al.*, 2008; Liao and Reed, 2009] and it is therefore important for our understanding of population biology. Understanding of inbreeding depression is also important in breeding and maintenance of endangered species [Frankham, 1995; Hedrick and Kalinowski, 2000], especially in wild populations [Crnokrak and Roff, 1999].

Inbreeding depression can impact a variety of traits. It may affect individuals across different life-history stages [Liu *et al.*, 2014]. It can also manifest in different morphological traits, particularly in those associated with fitness [DeRose and Roff, 1999]. Inbreeding depression can affect the 'quality' of the individual through affecting its development or body size [Crow and Kimura, 1970; DeRose and Roff, 1999]. Since different traits can be associated with fitness in each of the sexes, effects of inbreeding depression may be sex-specific [Enders and Nunney, 2010; Mallet and Chippindale, 2011]. Overall, inbreeding depression tends to be greater in fitness-related life-history traits than in morphological traits [DeRose and Roff, 1999; Angeloni *et al.*, 2011], and is reported to be particularly severe in the early life-history stages [Husband and Schemske, 1996]. Commonly investigated traits include hatching success and juvenile survival [Fernandez *et al.*, 1995; Kempenaers *et al.*, 1996], and in some cases inbreeding depression can drastically reduce recruitment from nests of relatives (e.g. collared flycatchers, Kruuk *et al.* [2002a]; see Neaves *et al.* [2015] for a review of inbreeding fitness consequences). However, in order to avoid underestimation, inbreeding depression should ideally be measured across all life-history stages, as it can be severe later in life [Keller, 1998; Costa e Silva *et al.*, 2011; Keller *et al.*, 2008; Winn *et al.*, 2011]. Furthermore, the ability to detect the presence of inbreeding depression and its strength, particularly in adult traits, may depend on the estimates of inbreeding used [Huisman *et al.*, 2016].

Moreover, the severity of inbreeding depression may depend on the species, the characteristics of

an individual and/or on environmental conditions [Hedrick and Kalinowski, 2000; Saccheri *et al.*, 1996]. It may also depend on the population size [Angeloni *et al.*, 2011] and on the inbreeding history of the population [Pemberton, 2008]. For instance, in previously inbred populations purging might have occurred reducing the severity of inbreeding depression relative to that in a historically outbred population. (Note: Variation with environmental conditions and purging are further explored in Section 1.4: *Studies of wild populations* below).

1.2.2. Inbreeding avoidance and its possible mechanisms

The widespread occurrence of inbreeding depression has generated a long-held assumption that inbreeding should always be avoided, where possible, due to selection against inbred individuals [Blouin and Blouin, 1988; Pusey and Wolf, 1996] and hence an assumption that inbreeding avoidance should be adaptive. Implicit assumptions about the presence of inbreeding avoidance and its adaptive value could affect the interpretation of results [Tregenza and Wedell, 2000; Nelson-Flower *et al.*, 2012] in studies of dispersal, multiple mating and mate choice [Blouin and Blouin, 1988; Pusey and Wolf, 1996; Hatchwell *et al.*, 2000; Jamieson *et al.*, 2009; Bretman *et al.*, 2009; Tregenza and Wedell, 2000; Geffen *et al.*, 2011; Brouwer *et al.*, 2011]. Given that inbreeding avoidance is explored in detail in Chapter 2, below I give only a brief overview of potential mechanisms behind this phenomenon.

Inbreeding avoidance mechanisms can be passive and/or active, and both types can potentially be exhibited independently or in combination in a single species. These mechanisms include: dispersal, kin discrimination, divorce and extra-pair reproduction. While extra-pair reproduction is often proposed as a mechanism of inbreeding avoidance [Arct *et al.*, 2015], evidence for this hypothesis is mixed [Reid, 2015; Nakagawa *et al.*, 2015; Forstmeier, 2015]. I outline these mechanisms below.

Dispersal

Mating with relatives could be avoided through dispersal, especially through sex-biased dispersal - where one sex is considerably more dispersive, or where only one sex disperses [Pusey, 1987; Johnson and Gaines, 1990]. If offspring disperse from their natal territories before they reach sexual maturity and breed, they will reduce the probability of mating with relatives. In some species where dispersal is uncommon the sexual maturation of offspring may be delayed [Wolff, 1992]. In the case of dispersal there is no need for kin discrimination - it is sufficient to reduce the encounter rate of relatives [Part, 1996; Szulkin and Sheldon, 2008; Lebigre *et al.*, 2010]. In systems where there is little or no dispersal, or where despite the dispersal individuals tend to associate with kin, other mechanisms may be needed to avoid inbreeding.

Kin discrimination

Inbreeding avoidance can also be effected through active discrimination against relatives as potential mates [Penn and Potts, 1999]. This could happen through pre-copulatory and post-copulatory processes. Pre-copulatory kin discrimination can be direct or indirect. During direct discrimination individuals are able to differentiate between relatives and non-relatives based on their phenotypes, for instance based on odour cues associated with major histocompatibility complex (MHC) [Penn and Potts, 1999] or by their calls [Price, 1999]. Indirect discrimination relies on familiarity (e.g. recognition of nest mates) [Blouin and Blouin, 1988; Pusey and Wolf, 1996]. Post-copulatory processes revolve around cryptic female choice, where females choose sperm from unrelated males over that of relatives [Bretman *et al.*, 2004, 2009; Gasparini and Pilastro, 2011; Tregenza and Wedell, 2000, 2002].

Divorce

Inbreeding avoidance through divorce may occur in systems with and without active kin discrimination. Kin discrimination of course allows differentiation between related and unrelated conspecifics, but individuals can obtain clues about the relatedness of their social partners in other ways too. For instance, they may divorce when hatching failure is high [Kempenaers *et al.*, 1998], as high levels of failure are indicative of genetic similarity and/or inbreeding depression. Therefore, by assessing the reproductive success with a specific partner, individuals may be able to assess the quality and compatibility of that partner. Divorce may involve the social pair only, for instance when the social pair is composed of related individuals [Walters, 1990], or the divorcing individual may move away from an entire family group to avoid potential future inbreeding [Hatchwell *et al.*, 2000].

Extra-pair reproduction (EPR)

Another possible mechanism of inbreeding avoidance is extra-pair reproduction (infidelity) [Pusey and Wolf, 1996; Blomqvist *et al.*, 2002; Tarvin *et al.*, 2005], where individuals cheat on their social partners and mate outside of the social pair. This strategy is potentially particularly important in systems where the choice of social partners is constrained and individuals may be forced to socially pair with relatives [Jennions and Petrie, 2000; Tregenza and Wedell, 2000; Griffith *et al.*, 2002; Kempenaers, 2007], regardless of the severity of inbreeding depression.

While extra-pair reproduction is often proposed as a mechanism of inbreeding avoidance, evidence for this hypothesis is mixed [Kempenaers, 2007; Szulkin *et al.*, 2013]. Several studies attempted to investigate the link between inbreeding and extra-pair reproduction: for instance, in red-winged fairy-wrens, western sandpipers, Kentish plovers, common sandpipers and blue tits, EPR seems to lead to inbreeding avoidance [Blomqvist *et al.*, 2002; Foerster *et al.*, 2003; Brouwer *et al.*, 2011], while in ground tits and meerkats it does not [Wang and Lu, 2011; Leclaire *et al.*, 2013]. Extra-pair reproduction is discussed in more detail below (Section 1.3),

and investigated as a potential inbreeding avoidance mechanism in Chapter 2.

1.2.3. Mating strategies: should individuals always avoid inbreeding?

Although the negative consequences of inbreeding depression generate a common expectation of inbreeding avoidance [Blouin and Blouin, 1988; Pusey and Wolf, 1996], this may not always be the case. This area of research has seen renewed interest in the last few years, with a number of discussion papers and theoretical models developed to address the circumstances under which inbreeding avoidance, preference and random-mating (sometimes referred to as inbreeding 'tolerance') should be expected (e.g. Kokko and Ots [2006]; Szulkin *et al.* [2013]; Cheptou and Donohue [2011]; Puurtinen [2011]; Duthie and Reid [2015]). For instance, under certain conditions the costs of inbreeding may be so low that there will be no selection against inbreeding [Waser *et al.*, 1986; Parker, 1979; Smith, 1979; Parker, 2006]. Mating with relatives may even have its advantages, as it allows the individuals to pass on more copies of parental genes to their offspring (more copies identical by descent; Waser *et al.* [1986]). This means that individuals may gain inclusive fitness benefits through inbreeding [Lande and Schemske, 1985; Charlesworth, 2006; Lehmann and Perrin, 2003; Kokko and Ots, 2006; Puurtinen, 2011], and these benefits may be high enough to offset the negative effects of inbreeding depression [Waser *et al.*, 1986; Parker, 1979; Smith, 1979; Parker, 2006]. Additionally, inbreeding avoidance mechanisms could be costly [Jamieson *et al.*, 2009; Kokko and Ots, 2006] and it may be difficult to establish whether a behaviour evolved as an inbreeding avoidance mechanism, or if it evolved for reasons unconnected to relatedness and the reduction in inbreeding is just a by-product of other processes.

While inbreeding avoidance has been documented across multiple species [Arct *et al.*, 2010; Brouwer *et al.*, 2011; Geffen *et al.*, 2011; Lemaitre *et al.*, 2012], it is not always found [Keller and Arcese, 1998; Foerster *et al.*, 2006]. In some cases individuals mate at random with respect to relatedness [Jamieson *et al.*, 2009; Szulkin *et al.*, 2009; Rioux-Paquette *et al.*, 2010]; or preferentially mate with relatives (inbreeding preference, [Thunken *et al.*, 2007; Wang and Lu, 2011; Stopher *et al.*, 2012]). Mating strategies are also expected to be context-dependent [Kokko and Ots, 2006; Szulkin *et al.*, 2013]. For instance, inbreeding may be more common in systems with sequential, rather than simultaneous, matings [Kokko and Ots, 2006]. It may also be more beneficial to inbreed than to forgo reproduction completely: for example, in collared flycatchers, rates of inbreeding increase later in the breeding season [Kruuk *et al.*, 2002a]. Moreover, trade-offs between costs and benefits of inbreeding could be sex-specific, as opportunity costs are expected to be higher for females than for males [Parker, 1979, 2006; Waser *et al.*, 1986]. Such variation in inbreeding strategies could indicate that the life histories and ecologies of species affect constraints on mate choice [Keller and Arcese, 1998; Jennions and Petrie, 2000; Jamieson *et al.*, 2009; Kingma *et al.*, 2013] and hence are also likely to affect relationships between inbreeding/relatedness and infidelity.

1.3. Extra-pair reproduction

The development of molecular techniques allowing paternity assignment has shattered the notion that the majority of bird species are monogamous. The study of mating systems now includes two components, the social mating system and the genetic mating system. The majority of bird species (90%) are socially monogamous [Clutton-Brock, 1991], but genetic promiscuity - extra-pair reproduction - has been shown to be common across bird taxa [Moller and Ninni, 1998; Cockburn, 2006; Griffith *et al.*, 2002]. The study system used in this thesis, the superb fairy-wren (*Malurus cyaneus*), is a case in point, as it was initially used as a textbook example of altruism and kin selection, but it was later discovered that the vast majority of broods contain extra-pair offspring [Rowley and Russell, 1997; Mulder *et al.*, 1994; Dunn and Cockburn, 1999]. The term 'extra-pair offspring' refers to any offspring produced by a female mating with a male ('extra-pair male') outside of her social pairing.

Rates of extra-pair paternity (EPP) are highly variable, both across species and within species. On the species level they can be anywhere from zero to nearly 70% of individuals, with an average rate of 11% across avian systems [Griffith *et al.*, 2002]: fairy-wrens (Maluridae) deserve a mention as they have the highest rates of infidelity of any bird family [Cockburn *et al.*, 2016]. Within-species variation is present both within populations (e.g. annual EPP variation in bluethroat (*Luscinia svecica*) is 7-33% [Johnsen and Lifjeld, 2003]) and between populations (e.g willow warbler (*Phylloscopus trochilus*) [Gyllensten *et al.*, 1990; Bjornstad and Lifjeld, 1997]). This variation in extra-pair paternity levels can be due to variation in social and ecological conditions [Neudorf, 2004; Schmoll, 2011]. (Note: these are explored further in Section 1.4: *Studies of wild populations* and Section 1.5: *Cooperative breeding and the role of social environment* below).

Extra-pair reproduction strategies may not only be species-specific, but may also be sex-specific. It was initially thought that extra-pair matings would be more beneficial to males and that multiple mating should be avoided by females. Males may attempt to coerce females into mating [Alatalo *et al.*, 1987; Morton, 1987; Jones *et al.*, 2012]. However, there is evidence from multiple species that females may tolerate extra-pair copulations, and even actively solicit them. Examples of species in which females solicit EPCs include blue tits [Kempenaers *et al.*, 1992], northern fulmars [Hatch, 1987], tree swallows [Lifjeld and Robertson, 1992], and zebra finches [Birkhead *et al.*, 1988], to name a few.

Despite extensive research in this area over the past several decades there is still no agreement about the function of extra-pair reproduction [Westneat and Stewart, 2003]. Many researchers argue that EPP is adaptive [Griffith *et al.*, 2002], but other ideas have emerged [Eliassen and Jorgensen, 2014; Forstmeier *et al.*, 2014]. It has been proposed that at the most basic level extra-pair reproduction may be a way of allowing the female to mate with a preferred male, especially in circumstances when choice of social partner is constrained [Brooker *et al.*, 1990; Pusey and Wolf, 1996; Eimes *et al.*, 2005]. Therefore, the female may obtain indirect genetic

benefits through extra-pair reproduction - she may for instance mate with a more genetically dissimilar male in order to avoid the costs of inbreeding depression. The study species used here, the superb fairy-wren, is an example of a system with limited social mate choice. Dispersal is female-biased and young females that fail to find a vacancy to settle on, die [Mulder, 1995]. It is therefore likely that the social mate the female settles with is not her optimal choice. Divorce and/or extra-pair reproduction could therefore be used by females to mate with the male of their choice.

1.4. Studies of wild populations

Studies of wild populations may be particularly informative in investigations of context-dependent mate choice and inbreeding-infidelity dynamics, as they allow conspecific interactions under a range of environmental conditions. In this section, first, I draw attention to how environmental conditions may affect extra-pair reproduction, and then identify why studying inbreeding in wild systems is a crucial addition to studies of laboratory and captive populations.

1.4.1. Infidelity under natural conditions

It is difficult to create natural mate choice under laboratory conditions, particularly for vertebrate species. In the wild, spatiotemporal patterns [Canal *et al.*, 2012] and environmental conditions can influence the distribution and availability of conspecifics. In turn, this may affect the pool of potential mates available to an individual, and thus impact on mating patterns. Schlicht *et al.* [2015] shows that breeding distance and density influence extra-pair pairings and argues that spatial setting of infidelity needs more attention.

To illustrate, population density is expected to increase with habitat quality and extra-pair reproduction could be affected through such variation in density [Mayer and Pasinelli, 2013]. This is because encounter rate increases with higher density, which may translate into higher availability of potential extra-pair mates [Birkhead, 1978; Westneat and Sherman, 1997]. Overall, there seems to be a positive association between population density and extra-pair paternity rates [Westneat and Sherman, 1997; Griffith *et al.*, 2002]. Furthermore, it is not purely a numbers game: available mates will not all be equal; they may differ in quality, and crucial characteristics, such as relatedness to the focal individual.

Beyond the number of potential mates, the availability and abundance of resources may also play a role in extra-pair reproduction. Female infidelity leads to paternity uncertainty, potentially leading to reduction in paternal care. According to the 'constrained female' hypothesis, females may be able to increase their infidelity when resources are abundant [Mulder *et al.*, 1994; Gowaty, 1996, 1997]. There is some evidence to support this; for instance, in serins the likelihood of extra-pair paternity is higher in territories with plentiful food [Hoi-Leitner *et al.*, 1999].

It has also been proposed that habitat type, structure and complexity may influence the difficultly of mate guarding: males in dense habitats may find it harder to spot incoming potential extra-pair mates and to defend their territories - and females - against such intruders [Sherman and Morton, 1988; Westneat and Sherman, 1997; Westneat and Stewart, 2003]. However, empirical evidence to support this is mixed, and Biagolini-Jr *et al.* [2017] failed to find support for a link between habitat complexity and infidelity in their recent comparative study.

Weather and climatic conditions may also play a role beyond their influence on the abundance of resources. For instance, cold weather may affect the amount of energy that individuals can allocate to extra-pair reproduction [Hoset *et al.*, 2014]. Bichet *et al.* [2016] link climate change to production of a higher proportion of extra-pair litters in Alpine marmots; and in blue-footed boobies females avoid participating in EP courtship during El Niño conditions (as warm waters increase the costs of behaviours associated with infidelity) [Kiere and Drummond, 2016].

1.4.2. Inbreeding under natural conditions

Inbreeding depression is well studied in captive and laboratory populations [Charlesworth and Charlesworth, 1987] and such studies can be particularly helpful in advancing our understanding of the genetic basis of inbreeding. However, magnitude of inbreeding depression can vary with environmental conditions [Jamieson *et al.*, 2003; Keller *et al.*, 2002; Marr *et al.*, 2006]. Even though it might be difficult to assess the interactions between environment and inbreeding depression [Pemberton *et al.*, 2017], there is evidence that inbreeding depression tends to be more severe in harsher, more stressful environments [Armbruster and Reed, 2005; Szulkin and Sheldon, 2007; Fox and Reed, 2010]. Furthermore, bottlenecks may lead to purging of deleterious mutations from a population, effectively reducing the negative consequences of inbreeding depression. This is particularly important for captive populations, which may lose genetic variation though purging over relatively short periods of time [Briscoe *et al.*, 1992] and therefore expression of inbreeding depression and inbreeding strategies may be weakened in these populations. Therefore, laboratory populations may not be representative of processes happening under natural conditions, and may underestimate the effects of inbreeding depression [Crnokrak and Roff, 1999].

Long-term studies carried out on large wild populations are necessary in order to investigate the consequences of inbreeding for the fitness of wild individuals and the strength of selection under varying environmental conditions. However, collecting data necessary to investigate inbreeding in the wild is time-consuming and difficult. Intensive monitoring of individuals over multiple generations and collection of genetic information is required in order to reconstruct a genetically-informed pedigree necessary for this type of work.

Moreover, it is particularly hard to study the details of inbreeding and inbreeding depression in vertebrates, and while captive and zoo populations provide some insight [Cassinello, 2005; Kalinowski *et al.*, 2000; Laikre *et al.*, 1996], there are relatively few detailed studies of

inbreeding in wild populations. Furthermore, many of the studies of wild vertebrates focus on island populations (for example: song sparrows [Keller, 1998]; Soay sheep [Coltman *et al.*, 1999]; red-cockaded woodpeckers [Daniels and Walters, 2000]; Takahe [Jamieson *et al.*, 2003]). These populations tend to be isolated and small - inbreeding is expected to be higher in such populations. Studies of populations with different structure and characteristics will be useful in advancing our understanding of inbreeding in the wild.

⋆⋆⋆

Inbreeding, inbreeding depression and infidelity may vary with temporal and spatial variation in environmental conditions, including varying with the social environment. Many studies consider the effects of the environment, but do not explicitly consider conspecifics to be a part of that environment. However, even individuals not directly involved in the mating may be able to influence reproductive decisions and outcomes. I will therefore consider the role of social environment in inbreeding and infidelity - the next section introduces this concept.

1.5. Cooperative breeding and the role of social environment

Interactions between conspecifics can influence morphology and physiology, as well as behaviours. In this thesis I use the phrase "social environment" as an all-encompassing term for the number and characteristics of conspecifics with whom the focal individual interacts (unless otherwise stated). The social environment during the early life and development of an individual can affect this individual's phenotype and behaviour later on [West-Eberhard, 2003]. For example, interactions with conspecifics could affect an individual's social competence [Taborsky and Oliveira, 2012; Branchi, 2009], aggression levels [Veenema, 2009] or morphogenesis [Pfennig and Collins, 1993]. This phenotypic plasticity plays an important role in the evolution and ecology of a species. Moreover, social environment can also be important on a much shorter timescale, affecting an individual's behaviour and even its morphology in 'real time' [Chapman *et al.*, 2008].

Social environment may be particularly important in social species [Branchi, 2009; Veenema, 2009] and in systems where relatives frequently interact. The importance of relatedness in reproductive and social biology is highlighted by the extensive use of kin selection theory in these areas of research. Since individuals can gain inclusive fitness benefits by assisting kin, relatives may, for example, exhibit more tolerance towards each other than do unrelated individuals [Carazo *et al.*, 2014, 2015].

Parents of many species cooperate when raising young as they both care for the offspring. There may be some conflict over the contributions of both parties to offspring-rearing [Trivers, 1972;

Clutton-Brock, 1991]. For instance, female infidelity leads to paternity uncertainty, in the light of which it may be adaptive for the male to reduce the levels of provided care. Ideally, each parent would want to put minimal effort in. However, if the overall care is reduced too much, offspring will suffer and might not even survive. Thus, in many species, there is some level of negotiation over expended effort and potential (partial) compensation when one of the parties reduces their effort ("negotiation models" [McNamara *et al.*, 1999, 2003; Johnstone and Hinde, 2006; Johnstone, 2011; Lessells and McNamara, 2012]). In many species more than two adults are engaged in raising offspring, and in some species more than two adults are necessary in order to successfully raise offspring (e.g. white-winged chough [Heinsohn, 1992]).

Relatives commonly interact in cooperatively-breeding species. In these cases, the relatedness of individuals interacting within a social group may be much higher than the average relatedness between any two random individuals within the population. Cooperatively breeding species are species where the breeding pair is assisted by conspecifics during their breeding attempts (Emlen [1991]; although several definitions have been used in the literature, see Hatchwell [2009]). These conspecifics are often referred to as "helpers". There is a wide variation in such cooperative systems. They can be facultative or obligate [Du Plessis *et al.*, 1995]. Helpers may be sexually mature and reproductively active (e.g. dunnocks [Davies, 1992]) or non-breeding (e.g. Florida scrub-jays [Townsend *et al.*, 2011]). In some systems both sexes help, while in others only one does [Riehl, 2013]. Helpers can also perform a wide range of tasks including territory defence; assistance with nest building and maintenance; sentinel behaviour; and babysitting duties to name a few (e.g. Santema and Clutton-Brock [2013]; Clutton-Brock *et al.* [2000]). Most crucially they provide additional offspring care in terms of food provisioning.

Such additional care may be relevant to both inbreeding and infidelity. For example, care provided by helpers could mitigate the negative effects of inbreeding depression and allow inbred offspring to survive and thrive (I investigate this in Chapter 2). As a corollary, it is even possible that helpers could facilitate higher rates of inbreeding. In terms of infidelity, the constrained female hypothesis states that females with more resources should exhibit higher rates of extra-pair paternity [Mulder *et al.*, 1994; Gowaty, 1996, 1997]. It could therefore be expected that females assisted by helpers cheat more on their social partners than unassisted females (explored in Chapters 2 and 3). Cooperatively-breeding groups are often comprised of relatives, but the role of interactions between relatedness and social environment in mate choice is not well understood (Chapter 3). It is important to investigate how these affect mate choice, but also to examine their consequences for the produced offspring (Chapters 2 and 5).

1.6. Study Species

1.6.1. Life-history and ecology of superb fairy-wrens

Superb fairy-wrens (*Malurus cyaneus*) are small (~10 g, ~120 mm in length, half of which is tail) multi-brooded Australian passerine birds from the Maluridae family. Adult superb fairy-wrens show sexual dimorphism and sexes can be easily identified in the field (Box 1). They are weak flyers [Rowley and Russell, 1997], foraging on the ground and shrubs. Their diet consists predominantly of arthropods, although stomach contents have also revealed the presence of seeds [Rowley, 1965; Barker and Vestjens, 1990].

Superb fairy-wrens are sedentary and live on year-round territories, with territory boundaries maintained throughout the year (although fusions and fissions of territories do happen (A. Cockburn, pers. comm.)). Each territory has a socially dominant male and a single breeding female, but might also have several subordinate individuals inhabiting the territory [Cockburn *et al.*, 2003]. The subordinate individuals on the territories are always male as fledgling dispersal is female-biased: young males are tolerated by the adults and philopatric [Cockburn *et al.*, 2008b], while young females are forced to leave the territory by the breeding female [Mulder, 1995]. Dispersing females either settle on a vacant territory or die (65-75% mortality [Mulder, 1995]): they never breed on their natal territory, even if the dominant female on that territory dies [Cockburn *et al.*, 2008b].

The majority of philopatric subordinate males stay on their natal territory till they die [Cockburn *et al.*, 2008b], and if they disperse, they usually move to an adjacent territory (95% of dispersing males [Cockburn *et al.*, 2008b]). Subordinates will always disperse when there is a breeding vacancy (i.e. an unpaired female) on a territory close by [Pruett-Jones and Lewis, 1990]. If a vacancy is not available the males become helpers and form stable queues for dominant status [Cockburn *et al.*, 2008b]. Such helpers are present during ~50% of all breeding attempts [Cockburn *et al.*, 2008b], making superb fairy-wrens facultative cooperative breeders.

Females build nests without any help, but all group members assist with the raising of the brood [Rowley, 1965; Pruett-Jones and Lewis, 1990]. Breeding season can span August-March. Due to high levels of predation, particularly at the egg-stage, females initiate up to eight clutches within one season, with an average of three eggs per clutch [Mulder *et al.*, 1994], fledging offspring from up to three broods [Mulder, 1995]. Young fledge ~12 days after hatching and are dependent on adults for food for four weeks after fledging [Mulder, 1995]. More nestlings hatch and fledge when the breeding pair has the support of the helpers [Rowley, 1965], suggesting that the presence of helpers affects the social environment experienced by the offspring (effects of helpers on offspring are explored further in Chapters 2 and 5).

As helpers tend to be sons from the previous broods, it is quite likely that the helper taking over the dominant position is pairing up with his mother; such pairings would produce highly inbred

offspring. In those cases there are no matings between the newly formed mother-son dominant pair; all offspring are extra-pair, suggesting avoidance of nuclear family inbreeding [Cockburn *et al.*, 2003]. I explore inbreeding, inbreeding depression and inbreeding avoidance in Chapter 2.

Extra-pair reproduction is not limited to mother-son social pairings, as despite being socially monogamous the superb fairy-wrens have one of the highest known rates of extra-pair paternity of any species studied to date, with extra-group males dominating parentage. Here, I test two hypotheses that could help explain such high infidelity rates: the inbreeding-avoidance hypothesis (Chapter 2) and the constrained female hypothesis (Chapter 3).

Throughout the year males participate in courtship displays almost always directed at extra-group females (97%, Mulder *et al.* [1994]) and mostly directed at the females in immediately-adjacent territories (73%, Mulder *et al.* [1994]). Male displays do not result in copulations; instead, females solicit extra-pair copulations during pre-dawn forays to the territory of their preferred extra-pair males [Double and Cockburn, 2000]. The majority of extra-group mates live within one or two territories of the focal female territory [Double and Cockburn, 2003]. While males seem to preferentially visit and display to extra-group females in neighbouring territories, there are still aspects of the patterns of courtship behaviour that have not been investigated. In Chapter 4, I investigate whether the relatedness of the female affects the visit rate of males or their courtship display.

1.6.2. Study site and population

This work is based on data from a long-term study of the superb fairy-wren population in and around the Australian National Botanic Gardens, a collection of native Australian plants on the lower slopes of Black Mountain in Canberra, Australia (35°16 S, 149°06 E). This is therefore a managed site but comprised of natural vegetation, approx. 60 ha (expanded from approx. 40 ha in 2001; Cockburn *et al.* [2008b]), containing 60-90 fairy-wren territories/year. The study population is located within a much larger superb fairy-wren population of unmonitored birds.

This study population has been intensively monitored since 1988 [Cockburn *et al.*, 2003]. Almost all birds were individually marked with colour rings, and therefore it is possible to identify the individuals in the field and follow them throughout their lives. Social groups and their composition were monitored throughout the year (weekly census, see Cockburn *et al.* [2003] for details). Data on social pairings, individual characteristics, fate and reproductive performance were collected. Blood samples were also collected, allowing the determination of paternity through microsatellite-based parentage assignment (see Double *et al.* [1997b] and Supplementary Information for Chapter 2).

Chapters 2, 3 and 5 use data from the years 1988-2013. Chapter 4 combines elements of the long-term data with observational data I collected over two consecutive field seasons (September-October in 2015 and 2016).

BOX 1: plumage differences between males & females

Females are grey-brown with off-white chests, with a blue-green tint to their tails, reddish-brown beaks, loral stripes and rings around their eyes (a). Juveniles of both sexes initially have shorter tails (~10 mm) and resemble females (but without the green-blue tint in the tail [Rowley, 1965]) for the first four months after fledging, after which males develop dark blue tails and black beaks [Mulder, 1995]. Males in nuptial breeding plumage are striking blue and black, with dark blue tail, blue cap, ear tufts and mantle, and black bill, collar, back and loral stripe; as well as dark brown wings and off-white chest (d). Males in non-breeding plumage are similar to females, but they never lose the black rings around their eyes, black colour of the bill and loral stripes; they also keep their dark blue tails throughout the year (b). Photos: GK Hajduk

1.7. Thesis Overview

1.7.1. Thesis Contribution

In this thesis, I investigate context-dependent mate choice in a superb fairy-wren population in Canberra, Australia. I focus on the role of social environment and relatedness between individuals in mate choice, concentrating on extra-pair reproduction as the means of mate choice.

Although there has been much research conducted on this population over the past three decades, inbreeding has not been investigated and reasons for extremely high infidelity rates remain unclear. Given the scarcity of non-isolated wild populations with genetically-informed pedigree data, this thesis will make a significant contribution to our understanding of inbreeding, inbreeding depression and mating strategies under natural conditions. It will also advance our knowledge of the causes of female infidelity and its variation. Finally, this thesis will provide valuable information about the consequences of relatedness and infidelity, as well as the consequences of social and environmental conditions, on the performance of produced offspring.

1.7.2. Thesis Structure

This thesis consists of an introduction (Chapter 1), four independent data chapters (Chapters 2 - 5) and a discussion chapter (Chapter 6). Each of the data chapters is fully-fledged, with its own introduction and discussion.

Chapter 1 provides background on a range of topics explored in this thesis: inbreeding, inbreeding depression, inbreeding strategies (particularly inbreeding avoidance), mate choice and extra-pair reproduction (infidelity), as well as social environment and cooperative breeding. I also include a section about the life-history and ecology of the study species, summarising the work previously carried out in this system.

Chapter 2 sets up the thesis by quantifying inbreeding and infidelity rates, as well as inbreeding depression. It goes on to address several questions at the intersection of infidelity, relatedness and social environment. It is set up as a comprehensive test of the assumptions of the inbreeding-avoidance hypothesis, with extra-pair paternity treated as a potential means to inbreeding avoidance. This chapter uses long-term data (26 years), and has been published in *Evolution*. Additionally, the Supplementary Information (Appendix A) details the methodology used to assign paternity in this population.

Chapter 3 explores female infidelity in more detail by investigating the interaction between the social environment and relatedness. According to the constrained female hypothesis females

should exhibit higher rates of extra-pair reproduction when assisted by helpers. Additionally, helpers of varied relatedness may provide different levels of assistance, freeing the female from the constraint to different degrees. I tested the effects of the relatedness between the helpers and the breeding female on female infidelity. This chapter uses 26 years of the long-term data and will be submitted as a manuscript to *Behavioural Ecology*.

Chapter 4. While I have focused on female mate choice in Chapters 2 and 3, Chapter 4 addresses how relatedness impacts male mate choice, by looking at whether males are interested in their mothers as mates. It also explores the relationship between mothers and sons in a different social context than the two previous chapters. Chapter 2 focuses on socially-paired mothers and sons, Chapter 3 considers females assisted by their sons (helpers), while this chapter covers the remaining scenario: males living away from their mothers. This study uses observational data of males visiting female territories and performing courtship displays that I collected over two two-month field seasons in 2015 and 2016.

Chapter 5 expands on work carried out in Chapter 2 with regards to consequences of mate choice for the produced offspring. Chapter 2 investigates inbreeding depression on two traits, nestling mass and fledgling survival. This chapter quantifies how much of the phenotypic association between mass and survival is driven by genetic, non-genetic, temporal and spatial effects, while taking into account the social environment.

Finally, in **Chapter 6**, I present a general discussion of the thesis results, placing them within the existing literature. I end by proposing avenues for future research, with a focus on understanding how spatiotemporal factors influence the dynamics between mating strategies and social environment in wild animal populations.

Chapter 2

Inbreeding, inbreeding depression, and infidelity in a cooperatively breeding bird

This chapter has been published:

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Author contributions and declaration: This chapter uses the long-term data, collected by multiple people since 1988, with particularly notable input from HLO, AC and NM. Planning of analyses: GKH, LEBK, AC, CAW. GKH performed all the data processing. GKH carried out the statistical analysis with guidance from LEBK. AC and CAW provided valuable dicussions. GKH and LEBK drafted the article. GKH revised the article with assistance from LEBK, CAW and AC.

GKH revised the article during two rounds of reviews in *Evolution* with guidance from LEBK. The text presented here is the final text as published in *Evolution*, but with minor formatting changes to form a more cohesive part of the thesis.

ABSTRACT

Inbreeding depression plays a major role in shaping mating systems: in particular, inbreeding avoidance is often proposed as a mechanism explaining extra-pair reproduction in socially-monogamous species. This suggestion relies on assumptions which are rarely comprehensively tested: that inbreeding depression is present, that higher kinship between social partners increases infidelity, and that infidelity reduces the frequency of inbreeding. Here, I test these assumptions using 26 years of data for a cooperatively-breeding, socially-monogamous bird with high female infidelity, the superb fairy-wren (*Malurus cyaneus*). Although inbred individuals were rare (~6% of offspring), I found evidence of inbreeding depression in nestling mass (but not in fledgling survival). Mother-son social pairings resulted in 100% infidelity, but kinship between a social pair did not otherwise predict female infidelity. Nevertheless, extra-pair offspring were less likely to be inbred than within-pair offspring. Finally, the social environment (the number of helpers in a group) did not affect offspring inbreeding coefficients or levels of inbreeding depression. In conclusion, despite some agreement with the assumptions that are necessary for inbreeding avoidance to drive infidelity, the apparent scarcity of inbreeding events and the observed levels of inbreeding depression seem insufficient to explain the ubiquitous infidelity in this system, beyond the mother-son mating avoidance.

2.1. Introduction

It is often expected that inbreeding depression should generate selection to avoid inbreeding [Pusey, 1987; Blouin and Blouin, 1988; Tregenza and Wedell, 2000; Szulkin *et al.*, 2013]. In particular, inbreeding avoidance has frequently been suggested as an explanation for female infidelity in socially-monogamous species. If individuals are likely to be socially paired with a relative, extra-pair paternity (EPP) may have adaptive advantages if it reduces rates of inbreeding [Blomqvist *et al.*, 2002; Foerster *et al.*, 2003]. The expectation that inbreeding depression is ubiquitous in diploids [Lynch and Walsh, 1998] and the difficulties inherent in explaining the occurrence of EPP [Griffith *et al.*, 2002; Westneat and Stewart, 2003; Arnqvist and Kirkpatrick, 2005; Forstmeier *et al.*, 2014] have jointly strengthened the appeal of this 'inbreeding-avoidance hypothesis' for the occurrence of extra-pair reproduction. However, quantifying the relevant parameters in empirical studies is challenging, and so our understanding of several key aspects of the interplay between inbreeding and infidelity in natural populations is still limited. Here, I use data from a long-term study to investigate the effects of exceptionally high rates of extra-pair paternity on inbreeding and inbreeding depression in a passerine bird.

Three assumptions are necessary to support the notion that extra-pair mating occurs to facilitate inbreeding avoidance: (1) that inbreeding depression is present; (2) that infidelity increases with kinship to social mate; and (3) that infidelity reduces the chances of inbreeding. I emphasise that

whilst these assumptions are necessary for there to be adaptive benefits of inbreeding avoidance *via* EPP (i.e. these will not occur without the assumptions being met), they may not be sufficient (i.e. the assumptions being met does not inevitably guarantee the outcome). In particular, with regard to (1), the existence of inbreeding depression may not inevitably select for inbreeding avoidance because of the potential inclusive fitness benefits of inbreeding (through increased reproductive success of relatives; e.g. Bengtsson [1978]; Parker [1979]; Kokko and Ots [2006]; Duthie and Reid [2015, 2016]). *Increased*, rather than decreased, rates of inbreeding *via* extra-pair paternity may even be adaptive if inbreeding depression is sufficiently mild [Lehtonen and Kokko, 2015]. Further, the overall benefits of inbreeding avoidance will also depend on the potential costs of any avoidance mechanisms [Koenig *et al.*, 1999; Lehmann and Perrin, 2003]. The selection pressures on the alternative mating strategies of inbreeding avoidance, inbreeding preference or random mating will therefore depend on the relative magnitudes of inbreeding depression, the benefits to inclusive fitness of inbreeding, and the costs of inbreeding avoidance [Szulkin *et al.*, 2013; Duthie and Reid, 2016; Duthie *et al.*, 2016a]. However, in setting out a necessary (if not sufficient) set of conditions, assumptions (1) - (3) provide a useful framework for evaluating the plausibility of inbreeding avoidance *via* extra-pair paternity.

Studies of wild populations will be especially valuable for evaluation of mating patterns and inbreeding, because laboratory studies may not be able to recreate natural patterns of mate choice, and also because inbreeding depression may change with environmental conditions [Crnokrak and Roff, 1999; Joron and Brakefield, 2003; Szulkin and Sheldon, 2007]. Studies to date have provided evidence from wild populations for each of the three assumptions outlined above. For example, Keller and Waller [2002] review evidence for inbreeding depression in the wild (assumption 1); Leclaire *et al.* [2013] and Arct *et al.* [2015] review evidence across species for relatedness to the social mate increasing EPP (assumption 2); and Foerster *et al.* [2003] and Reid *et al.* [2015b] document reduced inbreeding coefficients as a result of infidelity in two passerine bird species. However, comprehensive tests of all three assumptions within a single study system are scarce. One notable exception is the work on a Canadian population of song sparrows (*Melospiza melodia*), where evidence combined across several papers covers all three assumptions (assumption 1: e.g. Taylor *et al.* [2010]; assumptions 2 and 3: [Reid *et al.*, 2015a,b,c]. However, the song sparrow study involves a small, isolated island population where high levels of relatedness between individuals are expected and observed. Equivalent studies of systems with other characteristics will therefore be required for any indication of the generality of these patterns.

The scarcity of comprehensive empirical studies of the role of extra-pair (EP) reproduction in facilitating inbreeding avoidance may be partially due to the inherent difficulty of quantifying inbreeding and inbreeding depression in the wild. Analyses of inbreeding require estimates of relatedness between individuals, but in socially-monogamous systems, social pedigrees based on the observed parental behaviour cannot provide accurate estimates of relatedness when infidelity is present, meaning that genetic information of some form is ideally required. Several studies of

associations between extra-pair mating and relatedness (assumption 2) have used genetic rather than social data, but these are typically based on assessing inbreeding from heterozygosity at a handful of molecular markers, typically microsatellites (e.g. Smith *et al.* [2005]; Foerster *et al.* [2006]). This is potentially problematic as heterozygosity at a small number of markers may be only weakly correlated with genome-wide heterozygosity: just as it is now clear that inbreeding depression cannot reliably be estimated from correlations between heterozygosity of a few markers and trait values [Balloux *et al.*, 2004; Slate *et al.*, 2004; Szulkin *et al.*, 2010], studies that use a low number of markers to test for inbreeding avoidance through EP matings may not be able to estimate relevant levels of relatedness sufficiently accurately. Furthermore, estimates of inbreeding and relatedness may be marker-dependent [Wang, 2014], and using the same markers to evaluate both paternity and heterozygosity may lead to false-positives when assessing the role of heterozygosity in mate choice [Wetzel and Westneat, 2009]. These issues mean that genetically-informed pedigrees and/or high-density genomic data are required for accurate estimates of relatedness, inbreeding and inbreeding depression [Pemberton, 2004; Harrison *et al.*, 2013], and hence, for accurate tests of the hypothesis of inbreeding avoidance through EP reproduction in the wild.

The dynamics of inbreeding, inbreeding depression and infidelity may also be shaped by the social environment of individuals, in particular the number and characteristics of conspecifics with which they interact [Koenig and Haydock, 2004]. In cooperatively breeding species that live in groups of closely-related individuals, group composition may affect mating patterns and change any effects of inbreeding depression on offspring development. The social environment may affect the chances that an individual inbreeds: the likelihood that relatives will be socially paired may be higher in cooperative breeders where close adult kin are tolerated in the social group, than in other social systems. The hypothesis of inbreeding avoidance through EP matings assumes that extra-pair partners will be less closely related than social partners. However, this may not be so if the closest available alternative mate is equally related - a scenario which can readily occur in cooperatively breeding groups. Thus, immediate social environment may play an important role in shaping inbreeding and infidelity patterns.

There is also a general expectation that inbreeding depression may vary with environmental conditions (e.g. Miller [1994]; Armbruster and Reed [2005]; Fox and Reed [2010]), although detecting inbreeding-environment interactions has been difficult in wild populations [Pemberton *et al.*, 2017]. I might thus also expect inbreeding depression to vary with social environment. Recent theoretical work suggests parents should invest more care into inbred offspring to counteract the reduced viability of such offspring [Duthie *et al.*, 2016b] - by extension, if assisted parents in cooperatively breeding species can rely on helpers to provide additional care for offspring, potentially lessening the effects of inbreeding depression, selection against inbreeding could be reduced. Whether this occurs in natural populations is not yet clear: for example, a recent study of inbreeding in meerkats [Nielsen *et al.*, 2012] found positive effects of helpers but negative effects of inbreeding depression on offspring growth, but did not find evidence that

helpers mitigated the negative effects of inbreeding depression. However, such associations may play an important role in the impact of social environment on inbreeding-infidelity dynamics.

In this paper, I investigate the intraspecific relationship between inbreeding and infidelity, and its interaction with the social environment. My study species, the superb fairy-wren (*Malurus cyaneus*), is socially monogamous but has exceptionally high levels of female infidelity, effectively making it the least faithful species of the least faithful bird genus [Cockburn *et al.*, 2016]. I used data from a long-term study of a wild population in south-east Australia, including a genetically-based pedigree to estimate levels of inbreeding and inbreeding depression.

Superb fairy-wrens are characterised by substantial variation in levels of cooperative breeding. About half (54.5%) of breeding attempts in the population involve just a single pair of breeding adults, whereas the other half are helped by up to four (exceptionally rarely five) male subordinates (or 'helpers'), frequently sons from previous breeding attempts (61.8% of all helpers are sons of the breeding female). This cooperative breeding increases the chances of social pairing between relatives: helper males form a stable queue, and may inherit the dominant position and thus pair socially with their mother [Cockburn *et al.*, 2008b]. Individual females will therefore experience different social environments, dependent on whether they are breeding just as a pair or are accompanied by helpers, and whether they are socially paired to their son or not.

The current study is motivated by the observation that mothers never produce offspring with their sons, either when socially-paired to their sons as the dominant pair on a territory or when the sons are acting as helpers to their mother [Cockburn *et al.*, 2003]. This suggests fairy-wrens use extra-pair reproduction to avoid close inbreeding. Furthermore, mother-son pairs show no behavioural interest in each other as potential mates (A. Cockburn, pers. comm.). However, whether the three assumptions underlying the inbreeding-avoidance hypothesis outlined above all hold, and whether inbreeding avoidance extends to other levels of relatedness, is not known. Recently, Brouwer *et al.* [2017] explored multiple possible explanations for extra-pair reproduction across the fairy-wren (Maluridae) family. In support of inbreeding-avoidance, studies of four other fairy-wren species reported higher infidelity when social partners are more closely related (splendid fairy-wrens, Brooker *et al.* [1990]; Tarvin *et al.* [2005]; red-winged, Brouwer *et al.* [2011]; red-backed, Varian-Ramos and Webster [2012]; purple-crowned, Kingma *et al.* [2013]). However, these conclusions are all based on estimates of relatedness from microsatellite markers, rather than pedigree-based knowledge of identity of close relatives. Furthermore, despite invoking the inbreeding-avoidance hypothesis, these analyses do not assess inbreeding depression for offspring traits (with the exception of Kingma *et al.* [2013] finding evidence of hatching failure) or the implications of infidelity for inbreeding levels in the offspring.

My aims in this study were five-fold. Firstly, I used 26 years of multi-generational genetic pedigree data to quantify the frequency of inbreeding in superb fairy-wrens, and to identify particular routes by which it might occur. I then tested the three assumptions of the hypothesis

that extra-pair reproduction facilitates inbreeding avoidance by: (1) assessing inbreeding depression in two offspring traits, nestling mass and fledgling survival; (2) testing whether females who were socially paired to a relative were more likely to be unfaithful, extending the previous documentation of 100% infidelity in mother-son pairings [Cockburn *et al.*, 2003] to consider all possible levels of kinship between social partners. Then, (3) I quantified the overall effects of infidelity on the probability of offspring being inbred, testing whether extra-pair offspring were less likely to be inbred than within-pair offspring, and comparing observed levels of inbreeding with those that would have occurred had females always been entirely faithful. Finally, I assessed the impact of the social environment on each of these four aspects, by considering whether effects were mediated by the number of helpers in all analyses.

2.2. Materials and Methods

2.2.1. Study system

The colour-banded population of superb fairy-wrens (*Malurus cyaneus*) in and around the Australian National Botanic Gardens, Canberra, Australia (35°16 S, 149°06 E) has been intensively monitored since 1988 [Cockburn *et al.*, 2003]. The study site measures ~60 ha, contains 60-90 territories/year, and is surrounded by unmonitored superb fairy-wren territories; the study population is thus a sample of a much larger population with free movement across its boundaries. In this paper I use data from the years 1988-2013.

All study population birds were censused throughout the year [Cockburn *et al.*, 2003], with data collected on group composition, social pairings, fates and reproductive performance of individuals. Females can successfully raise up to three broods in a single season (between August and March each year), with each brood containing 3-4 young [Cockburn *et al.*, 2008c]. However, because predation rates are high, as many as eight clutches may be initiated in a season [Cockburn *et al.*, 2008c]. Nestlings were banded 5-8 days after hatching. A blood sample was taken at the same time, and microsatellite genotyping was used to assign parentage to all individuals [Double *et al.*, 1997b]; for paternity assignment details see the Supplementary Information (SI).

Since I tested the association between the number of helpers and EPP rates, it is worth noting that the large majority of extra-pair paternity is extra-group (i.e. involving a male on a different territory, in a different social group). Helpers gain little paternity within their social group, although many gain substantial reproductive success through extra-group matings [Double and Cockburn, 2003]. In particular, helpers only gain within-group paternity when their mother is no longer the breeding female, because she has either died or divorced her social partner to move to another territory, and so has then been replaced as breeder by an unrelated female [Cockburn *et al.*, 2003]. Furthermore, if the breeding female is socially paired with her son, all other helpers on the territory will most likely also be her sons [Cockburn *et al.*, 2008b], leaving the female

with no outbred mating opportunities on the territory.

2.2.2. Pedigree reconstruction and quantifying levels of inbreeding

Eight exceptionally polymorphic microsatellite loci and a stepwise process were used to assign paternities while taking into account the structure of the population; this allows identification of sires with near 100% certainty. Further details are provided in the SI. Using the parentage data, I constructed a multi-generational pedigree for individuals sampled between 1988 and 2013: this pedigree had maximum depth of 15 generations. I estimated inbreeding coefficients (f) from the pedigree for individuals for whom the identities of both genetic parents and at least one grandparent were known ($n = 4431$). Note that inbreeding between distant relatives could have been underestimated for individuals with less complete pedigree data. I therefore provide details of the effect of restricting to higher numbers of known grandparents on the sample sizes and inbreeding rates in the SI (Table A.1).

For each social pair (i.e. for each territory, the breeding female and the dominant male - always the oldest male on the territory), I calculated a kinship coefficient (k_{soc}), and for each female-EP male pair that produced extra-pair offspring (EPO), I also calculated a kinship coefficient (k_{EP}). The kinship coefficient between two individuals is defined as the probability that homologous alleles sampled from two individuals are identical by descent [Wright, 1922], and is equal to the inbreeding coefficient of offspring that would be produced by these individuals. Throughout, I distinguish between the 'genetic father', meaning the male who sired a particular offspring, and the 'social father', meaning the male who was dominant on the territory at the time that the offspring was hatched, and who may or may not have been the genetic father.

The variables fitted in the statistical models described below varied depending on the model, thus sample sizes for individual models varied and are given alongside the model results. All analyses were carried out in *R* version 3.3.1 [Development Core Team, 2011]. See SI for general information on individual/parent numbers in the main dataset.

2.2.3. Inbreeding depression

I estimated inbreeding depression in nestling mass and in fledgling survival.

Inbreeding depression in nestling mass. I fitted a linear mixed effects animal model using the *R* package *ASReml-R* version 3 [Butler *et al.*, 2009], with `nestling mass` (continuous) as the response variable, with Gaussian errors. An animal model (i.e. incorporating an additive genetic effect; Kruuk [2004]) was fitted to avoid any potential bias of estimates of inbreeding depression by not accounting for heritable genetic effects [Becker *et al.*, 2016], and to provide an estimate of the heritability of nestling mass. Significance of fixed effects was assessed using Wald statistics (with a critical level of $p < 0.05$). **Fixed Effects:** The `inbreeding coefficient` of each individual was fitted to test for potential inbreeding depression. I also fitted: number of

`helpers` (as a three-level factor: 0, 1 and 2+, where 2+ level consisted mainly of 2 helpers with some pairs assisted by 3 or 4 helpers; Kruuk *et al.* [2015]); `brood size` (the number of nestlings, 3-5), to account for the variation in the amount of care provided to the individual nestlings; and `sex` of nestling (male, female), to account for differences in size between males and females. Additionally, `nestling age` at measurement (continuous, in days; as a quadratic function) was fitted because pragmatic considerations meant that nestlings were weighed at different ages (days 5-8) and hence at different stages of their development. I fitted a two-level factor '`1992`' (pre-1992, 1992+) to account for the introduction of a new weighing protocol in 1992, which changed the time of day at which nestlings were weighed [Kruuk *et al.*, 2015]. **Random Effects:** I fitted `nest ID` to account for any similarities across multiple offspring from the same brood; an `additive genetic effect` (with covariance structure determined by the pedigree) to test for covariance between relatives [Kruuk, 2004]; and a multi-level factor of `cohort` to represent inter-annual variation (1988-2013: the '2013' cohort incorporates nestlings from August 2013 through to March 2014 *etc*.). Finally, I represented intra-annual temporal variation across the breeding season by fitting a multi-level factor of `hatch date` interval (split into twelve two-week intervals, between 23 September and 15 March).

The above model was run using the *R* package *ASReml-R*, as the response required Gaussian errors. All the following models were run using the *R* package *MCMCglmm* [Hadfield, 2010] to allow for binomial errors. For all the *MCMCglmm* models the effective sample sizes for specific parameters varied due to autocorrelation, but I ensured that they were always above 1000. I considered terms to be statistically significant based on 95% CIs (credible intervals) not spanning 0 and *pMCMC* values (the number of simulations greater or smaller than 0 corrected for number of *MCMC* samples) calculated by *MCMCglmm* being < 0.05. Details of model settings, such as the number of iterations, burn-in, thinning interval and priors for each model can be found in the SI.

Inbreeding depression in survival. I investigated `survival` from fledging to independence (from 12 to 41 days; see the SI for details on how these bounds were chosen). I ran two generalised linear mixed effects animal models using the *MCMCglmm* package. I first tested whether inbreeding affected `survival`, and then investigated whether any effect of inbreeding acted through body `mass`, by including `mass` as a covariate in the analysis. I modelled `survival` as a binary (0/1) response variable, with binomial errors. **Fixed Effects:** `inbreeding coefficient`, number of `helpers`, `brood size` and an individual's `sex` were fitted as described above (with `nestling mass` as an additional covariate in one of the models). **Random Effects:** `nest ID`, `additive genetic effect`, `cohort` and `hatch date` interval were fitted as described above.

I added in an interaction between the `inbreeding coefficient` and the number of `helpers`, to test whether helpers could mitigate the effects of inbreeding depression (see SI).

2.2.4. Effects of kinship between social partners on infidelity

I tested whether patterns of EPP were associated with either `kinship` between social partners (k_{soc}) and/or social environment, specifically the number of `helpers` at the nest. I used the proportion of EPO in each brood (as an index of infidelity) to investigate factors affecting a female's likelihood of producing extra-pair young.

It is possible that inbreeding depression in early embryo/nestling survival could bias later estimates of the extent of EPP in pairs where social partners are related. If all WPO die due to inbreeding depression, the clutch will appear to be composed entirely of EPO and rates of EPP will be overestimated [Reid, 2015; Reid *et al.*, 2015b]. To assess whether such 'selective disappearance' affected my estimates, I tested whether kinship between the social partners affected clutch size and/or survival of nestlings prior to measurement age. I found that k_{soc} was not associated with either clutch size or survival to measurement (Table A.2). (Note that this analysis also provided a test of inbreeding depression in early survival, but that this test was indirect; highly accurate assessment of early inbreeding depression is difficult. See SI for details.)

When assessing EPP levels, cases of mother-son pairings required special consideration. As described above, both behavioural and genetic analyses indicate that mothers and their sons never mate, even when socially paired, suggesting strong inbreeding avoidance in these pairings. I therefore ran two versions of my models: (a) using all available data; and (b) excluding mother-son pairings. This allowed me to test whether the results were disproportionately affected by the special case of mother-son pairings, without restricting exploration of the effects of kinship and the social environment beyond the mother-son pairings.

To test the effect of `kinship` between social partners on infidelity I fitted binomial generalised linear mixed models in *MCMCglmm*, with the `proportion of EPO` in a brood (defined by the numbers of extra- *vs* within-pair offspring) as a response variable, and binomial errors; these models were by definition fitted at the level of the brood rather than individual nestlings. **Fixed Effects:** `kinship` (continuous) was fitted to test whether the probability of offspring being sired by an extra-pair male varied with the kinship between the female and her social mate (k_{soc}). The number of `helpers` (0, 1 and 2+) was fitted to test whether the social environment affected the probability of extra-pair reproduction. Additionally, the `mother's age` and the `social father's age` (two-level factors: one-year-old, older) were fitted to account for potential effects of differences in experience. **Random Effects:** `mother ID` and `social father ID` were fitted to account for the multiple observations on specific females and males (social fathers); `cohort` was fitted as above.

I then fitted both of the models of EPP rates with an interaction between the `kinship` and the number of `helpers`, to test for the role of social environment (see SI).

2.2.5. Effects of infidelity on inbreeding

This final section tested the consequences of EPP for the probability of offspring being inbred. Due to the high proportion of zeros (94.5%) amongst the estimated inbreeding coefficients, the inbreeding coefficient did not fit any standard distribution for a continuous covariate. I therefore used a two-step process: first I fitted a model with a binomial response of whether an individual offspring was inbred ($f > 0$) or not ($f = 0$), and then I fitted a model with a continuous response, but only considering inbred individuals ($f > 0$) (see Huisman *et al.* [2016] for a similar two-stage analysis).

The models were fitted excluding mother-son pairings to avoid any bias stemming from those special cases. Additionally, because the number of helpers affects rates of EPP (Cockburn *et al.* [2016] and references therein, and see below), I did not fit the number of helpers in these models, given the potential confounding effects between the two.

Step 1. I fitted a binomial generalised linear mixed model using *MCMCglmm*, with the `inbreeding status` of every offspring as a response (two-level factor: inbred *vs* outbred, where an inbred individual had $f > 0$). **Fixed Effects:** I fitted `within-pair status`, i.e. whether an offspring was the result of within-pair (WP) or extra-pair (EP) reproduction, as a two-level factor. **Random Effects:** `nest ID` and `cohort` were fitted as above.

Step 2. I tested what determined the magnitude of the inbreeding coefficient amongst those nestlings that were inbred, i.e. had $f > 0$. I fitted a linear mixed model using *MCMCglmm*, with log-transformed inbreeding coefficient as the response variable, and Gaussian errors. I used only the inbred individuals ($f > 0$) in this model. **Fixed Effects:** I fitted `within-pair status` (a two-level factor: WP *vs* EP). **Random Effects:** `nest ID` and `cohort` were fitted as above.

As a final step, I asked what level of inbreeding would have occurred if there had never been any extra-pair reproduction. To do this, I constructed an artificial social 'faithful' pedigree to represent the relatedness patterns which would have occurred had all females always been faithful and their social mates were always the genetic fathers of all their offspring. Comparing the inbreeding coefficients that would have resulted if this pedigree were real with the actual observed inbreeding coefficients allowed me to judge the overall impact of infidelity on inbreeding in the population.

In order for the *MCMCglmm* models to run satisfactorily, it was necessary to truncate the latent variables for models in this section (J. Hadfield, pers. comm.); see SI for details.

2.3. Results

2.3.1. Pedigree reconstruction and levels of inbreeding

Kinship between superb fairy-wren social partners (k_{soc}) ranged from zero to 0.25, with the latter category consisting entirely of pairings between mothers and sons; the mean k_{soc} across 863 unique social pairings was 0.0129 (*median* = 0). Only 10.5% of social pairings were incestuous ($k_{soc} > 0$), with mother-son pairings accounting for 4.2% of all social pairings (Table 2.1a).

Inbreeding appeared rare: of the 4431 offspring with at least one grandparent known, only 5.5% were inbred (i.e. had $f > 0$) (Table 2.1b). There were no individuals with $f = 0.25$ (as would result from reproduction between a parent and offspring or from a full-sibling pairing)[1]. The maximum inbreeding coefficient observed was $f = 0.125$, and occurred *via* a variety of routes (Table 2.1b). Tighter pedigree restrictions led to an increased overall frequency of inbreeding, due to tighter restrictions generally resulting in the exclusion of 'outbred' ($f = 0$) individuals (Table A.1). This increase was particularly prominent when maternal grandparents were included in the restriction: a requirement of at least three known grandparents corresponded to a frequency of inbred individuals of 18.2%.

2.3.2. Inbreeding depression on nestling mass and survival

Nestling mass declined with inbreeding coefficient, providing evidence for inbreeding depression (Table 2.2, Figure 2.1a): the mean observed mass for nestlings with $f = 0.125$ was ~10% lower than the mean observed mass for nestlings with $f = 0$ (see Table A.9 for predicted nestling mass across different f values). In line with previous results [Kruuk *et al.*, 2015], I also found that: males were heavier than females; nestlings from smaller broods were heavier than those from larger broods; those from broods assisted by helpers were heavier than those from unassisted broods; and, unsurprisingly, nestling mass increased with the age at which they were measured (Table 2.2). Additionally, the heritability of nestling mass was estimated as 18.9% (±3.2% SE). However, the social environment had no effect on the realisation of inbreeding depression ($p = 0.98$; Table A.3a).

In contrast, there was no evidence of inbreeding depression for survival, whether or not nestling mass was included in the model: the probability of a fledgling surviving to independence was not affected by its inbreeding coefficient. Heavier individuals did survive better (Figure 2.1b, Table 2.2), but none of the other variables modelled were associated with changes in survival (Table 2.2). As above, I found no statistical support for an interaction between the effect of inbreeding on nestling mass and the number of helpers (Table A.3).

[1] Since I carried out this analysis, individuals hatched after 2013 were genotyped using SNP data, providing the first evidence of nuclear family inbreeding event in this population.

Table 2.1. Distribution of kinship between social partners and of inbreeding coefficients. (a) Percentage of broods for which the social parents were relatives ($k_{soc} > 0$) (out of n = 1745 broods), then split into high, moderate or low levels of k_{soc}, and followed by details of individual cases where $k_{soc} \geq 0.125$; and (b) percentage of inbred offspring, considering the inbreeding coefficient ($f > 0$) at the individual level (out of n = 4431 individuals), then split into high, moderate or low levels of f, followed by details of individual cases where $f \geq 0.125$.

(a) kinship between social partners (brood level)		broods % (n)
all: $k_{soc} > 0$		10.5% (183/1745)
high: $k_{soc} \geq 0.25$		4.2% (73/1745)
moderate: $0.125 \leq k_{soc} < 0.25$		0.7% (12/1745)
low: $k_{soc} < 0.125$		5.6% (98/1745)
male social partner	female social partner	broods % (n)
high: $k_{soc} \geq 0.25$		
son	mother	4.2% (73/1745)
moderate: $0.125 \leq k_{soc} < 0.25$		
paternal half-brother	paternal half-sister	0.2% (4/1745)
maternal half-brother	maternal half-sister	0.2% (4/1745)
grandson	maternal grandmother	0.2% (4/1745)
(b) inbreeding coefficient (individual level)		individuals % (n)
all: $f > 0$		5.5% (245/4431)
high: $f \geq 0.25$		0.0% (0/4431)
moderate: $0.125 \leq f < 0.25$†		0.3% (14/4431)
low: $f < 0.125$		5.2% (231/4431)
male parent	female parent	individuals % (n)
moderate: $0.125 \leq f < 0.25$†		
paternal half-brother	paternal half-sister	0.09% (4/4431)
maternal half-brother	maternal half-sister	0.02% (1/4431)
grandson	paternal grandmother	0.05% (2/4431)
grandson	maternal grandmother	0.1% (5/4431)
paternal grandfather	granddaughter	0.05% (2/4431)

† for consistency with (a), I refer to "moderate" inbreeding as $0.125 \leq f < 0.25$, but in practice all individuals in this category were f = 0.125

Table 2.2. Test for inbreeding depression. Effects of inbreeding coefficient f on (a) nestling mass; and on survival from fledging to 41 days fitted (b) with nestling mass (corrected for change in protocol in 1992 and for nestling age at measurement) included as a covariate, and (c) without nestling mass included. There was no support for an interaction between the inbreeding coefficient and number of helpers, thus the interaction was dropped from the models and simple models are presented below; for models with interaction see Table A.3. (Note that the precise form of output differs for the *ASReml-R* model in (a) *versus* the *MCMCglmm* models in (b)/(c).)

	nestling mass (g)		**survival from fledging to 41 days**			
	(a)		(b) with mass		(c) without mass	
Fixed effects	**Estimate (SE)**	**P**	**Posterior mean (95% CI)**	**P**	**Posterior mean (95% CI)**	**P**
intercept	−3.83 (1.21)	**0.008**	-0.92 (-3.02, 1.18)	0.364	1.41 (0.14, 2.73)	**0.035**
1992 (1992+, pre-1992)		<**0.001**				
pre-1992	0.62 (0.12)					
nestling age	2.16 (0.35)	<**0.001**				
nestling age^2	−0.09 (0.03)	<**0.001**				
brood size	−0.05 (0.02)	**0.012**	-0.17 (-0.45, 0.13)	0.257	-0.20 (-0.48, 0.11)	0.194
sex (female, male)		<**0.001**				
male	0.15 (0.02)		-0.08 (-0.38, 0.18)	0.595	-0.02 (-0.30, 0.26)	0.881
mass			0.33 (0.07, 0.55)	**0.004**		
helpers $(0, 1, 2+)$		<**0.001**				
1 helper	0.09 (0.04)		0.26 (-0.24, 0.73)	0.278	0.29 (-0.16, 0.78)	0.223
2+ helpers	0.20 (0.04)		0.33 (-0.20, 0.93)	0.252	0.40 (-0.14, 0.91)	0.146
inbreeding coefficient	-3.64 (1.26)	**0.004**	-6.92 (-27.45, 13.83)	0.497	-5.33 (-24.35, 16.00)	0.590
Random effects	**Variance (SE)**		**Posterior mean (95% CI)**		**Posterior mean (95% CI)**	
nest ID	0.23 (0.01)		6.53 (4.68, 8.76)		6.37 (4.48, 8.31)	
hatch date	0.01 (0.01)		2.54 (0.60, 5.78)		2.67 (0.54, 5.89)	
cohort	0.01 (0.01)		0.30 (8.91^{-5}, 0.70)		0.32 (2.27^{-5}, 0.75)	
additive genetic effect	0.10 (0.02)		1.54 (0.17, 3.10)		1.48 (0.22, 3.00)	
residual variance	0.19 (0.01)		n/a		n/a	
Sample size	4167		3187		3200	

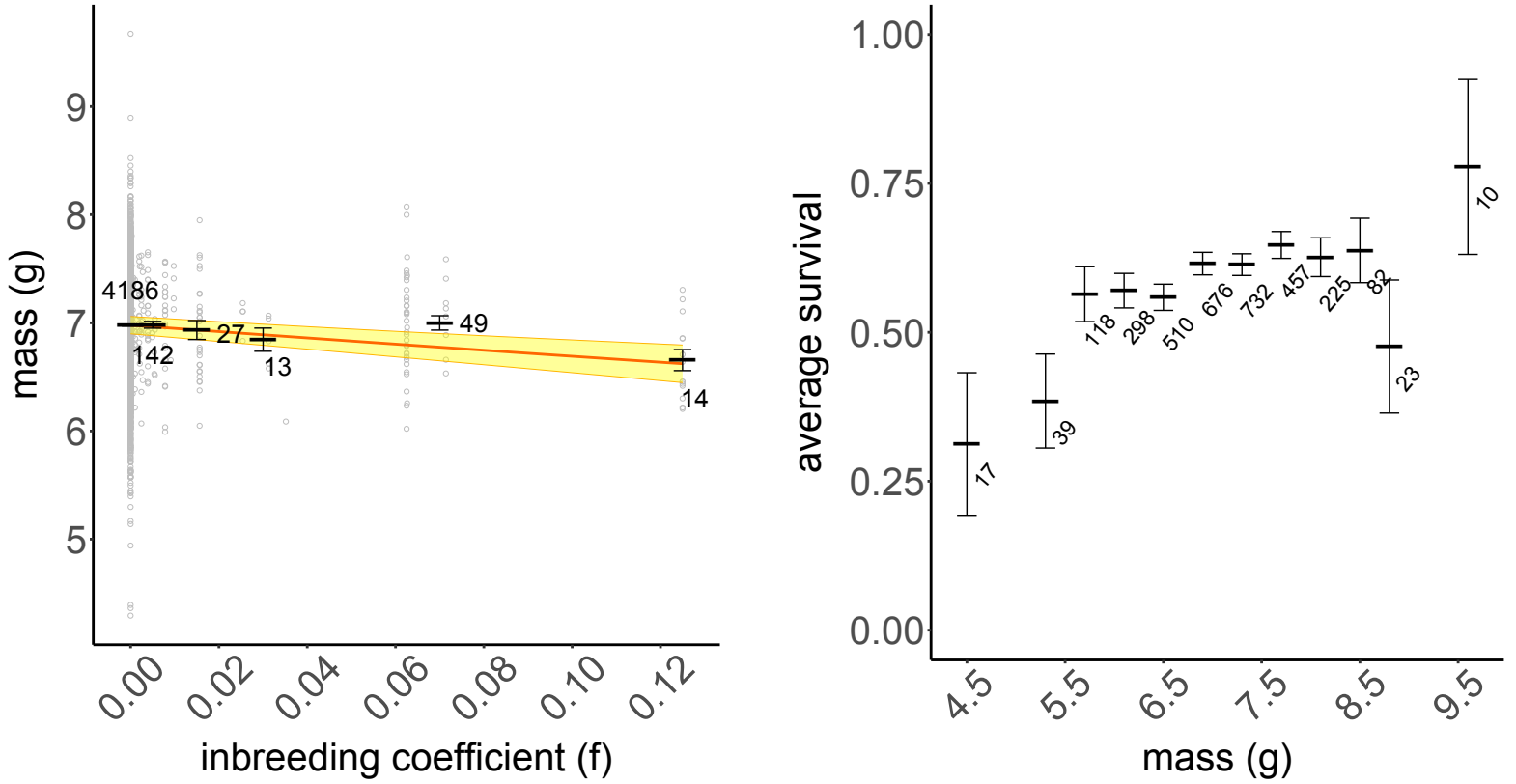

Figure 2.1. (a) Effects of inbreeding coefficient (f) on nestling mass. Mass was corrected for the change in protocol in 1992 and for the age of the nestling at measurement, with mean mass at day 7 being presented. Grey open circles represent the raw data, black dashes show means of data grouped into bins (0, between 0-0.01 non-inclusive of bounds, then 0.01-0.02, 0.02-0.04, 0.06-0.08, 0.12-0.13 with lower bound inclusive) with the group sample sizes indicated next to the groups (total n = 4431), and with error bars representing standard errors. The solid orange line represents the predictions from a linear mixed effects model, aligned with the intercept of the raw data, with shading around the line showing standard errors. (b) Effects of nestling mass on survival from fledging to 41 days. Dashes represent mean survival of individuals with nestling mass binned (3.9-5.1, then every 0.4, till 8.7-9.0, 9.0-10.2, lower bound inclusive; note that bins at the extremes are wider) with error bars showing standard errors and group sample sizes indicated next to the groups (total n = 3187).

2.3.3. Effects of kinship between social partners on the levels of extra-pair reproduction

Out of the 4431 individuals with known parents and at least one known grandparent, 2704 (61%) were extra-pair, and out of the total 1745 broods, 1445 (82.8%) had at least one extra-pair offspring.

Considering the full data-set including the mother-son pairings, the proportion of EPO in the brood increased with increasing kinship, k_{soc} (Figure 2.2, Table 2.3a). However, k_{soc} had no effect on EPO frequency when the mother-son pairings were removed (Figure 2.2, Table 2.3b), indicating that its effect on infidelity rates was driven by the special case of mother-son pairings, where infidelity was 100%. Outside of the mother-son pairings, the mean percentage of EPO per brood was 73.3%. The proportion of EPO also increased with increasing helper number, regardless of whether mother-son pairings were included in the model or not. However, the effects of k_{soc} were not modulated by the number of helpers (Table A.4). Mother's age and social father's age also had no effect in either model (Table 2.3).

Table 2.3. Effects of kinship between the social male and female and the effects of helpers on the proportion of extra-pair offspring in the brood. Models were run (a) on all data, including mother-son pairings; and (b) excluding mother-son pairings and any offspring produced by females socially paired to their sons.

	proportion of extra-pair offspring in the brood			
	(a) with mother-son		(b) without mother-son	
Fixed effects	**Posterior mean (95% CI)**	**P**	**Posterior mean (95% CI)**	**P**
intercept	0.47 (0.11, 0.84)	**0.017**	0.49 (0.13, 0.88)	**0.009**
mother age (1yo, older)				
older	0.16 (-0.12, 0.42)	0.248	0.13 (-0.15, 0.39)	0.356
social father age (1yo, older)				
older	-0.16 (-0.55, 0.20)	0.400	-0.13 (-0.53, 0.25)	0.522
helpers (0, 1, 2+)				
1 helper	0.53 (0.24, 0.82)	**0.004**	0.55 (0.23, 0.81)	**0.002**
2+ helpers	1.17 (0.78, 1.55)	**<0.001**	1.17 (0.82, 1.58)	**<0.001**
kinship	18.69 (13.65, 24.29)	**<0.001**	-1.19 (-11.04, 9.41)	0.807
Random effects	**Posterior mean (95% CI)**		**Posterior mean (95% CI)**	
mother ID	0.73 (0.33, 1.15)		0.69 (0.30, 1.09)	
social father ID	0.71 (0.33, 1.07)		0.72 (0.40, 1.12)	
cohort	0.02 (5.36^{-09}, 0.06)		0.02 (9.73^{-09}, 0.06)	
residual variance	1.85 (1.35, 2.33)		1.82 (1.38, 2.36)	
Sample size	1473		1421	

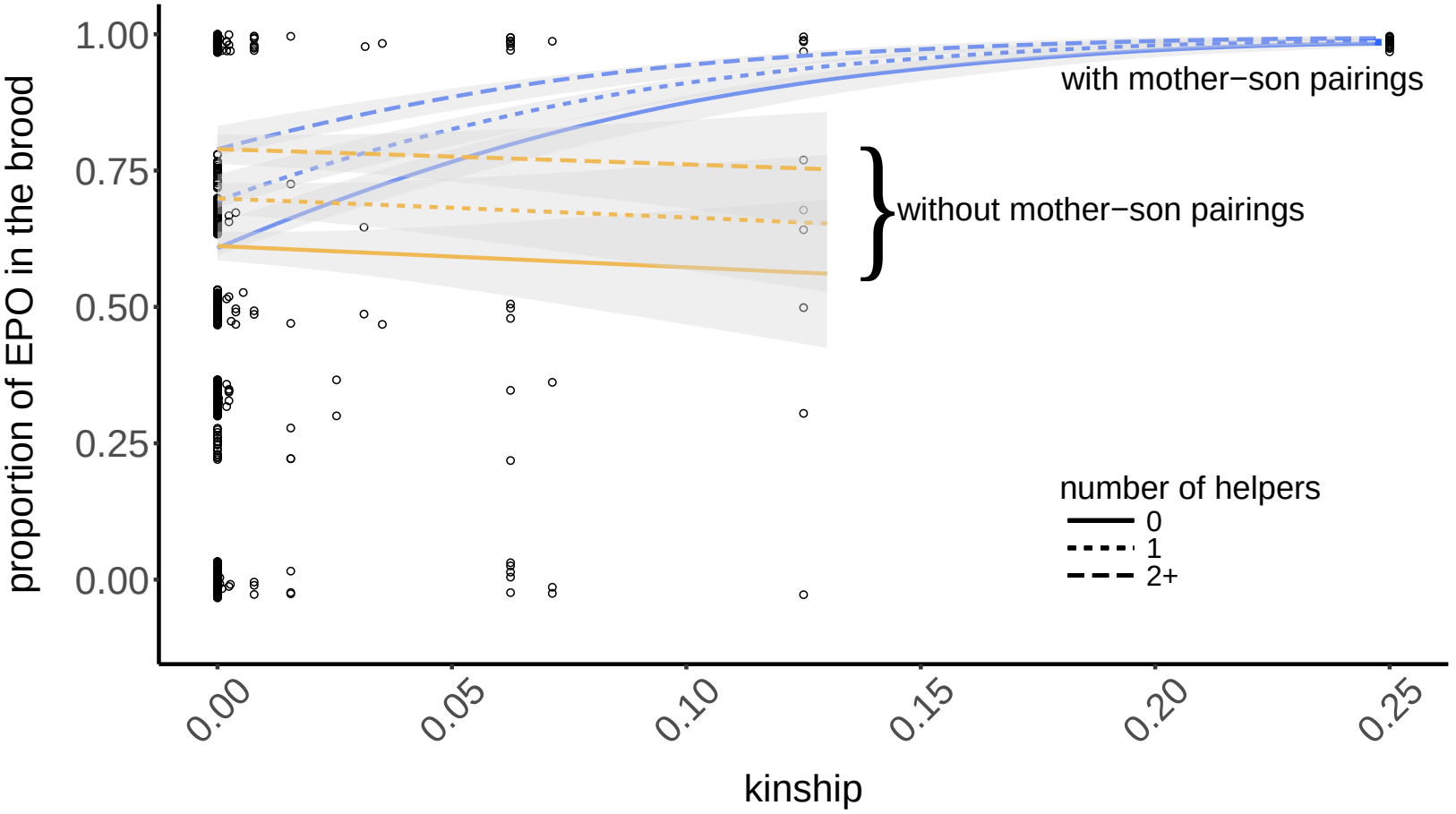

Figure 2.2. Effects of kinship between the social male and female on the proportion of extra-pair offspring (EPO) in the brood. Open circles represent raw data, which has been jittered to aid visualisation. Data are presented with mother-son pairings (blue lines), as well as without the broods produced by females socially paired to their sons ("without mother-son pairings"; orange lines). The lines represent model predictions from generalised linear mixed effects models, split by the number of helpers (0, 1 and 2+) to emphasise the impact of helpers, with shading around the lines showing standard errors.

2.3.4. Effects of infidelity on the probability of offspring being inbred

Kinship between females and their extra-pair partners (k_{EP}) ranged from zero up to 0.125 (Table 2.1a), with the highest value representing three extra-pair pairings that occurred between grandmothers and grandsons, and one extra-pair pairing between granddaughter and grandfather; the mean k_{EP} across 1197 unique extra-pair pairings was 0.0012 (*median* = 0). Infidelity reduced inbreeding: if all females had been faithful to their social partners throughout the study period, 10.4% of all individuals in the population would be inbred ($f > 0$) compared to the observed 5.5% (Table 2.4; Fisher's exact test: $p < 0.001$). Excluding mother-son pairings, these frequencies become 9.3% *vs* 4.5% (Fisher's exact test: $p < 0.001$).

Table 2.4 shows the frequency of inbred ($f > 0$) *vs* outbred ($f = 0$) individuals in relation to their extra-pair *vs* within-pair status: there were more inbred WPO than EPO (Fisher's exact test: $p < 0.001$). Moreover, this was the case regardless of pedigree restrictions applied (Table A.5). The mixed model confirmed that EPO were less likely to be inbred than WPO (Table 2.5), while the number of helpers had no effect on inbreeding status (Table A.8). These results were also consistent across different pedigree restrictions (Table A.6). Furthermore, the second step of this analysis showed that amongst inbred offspring ($f > 0$), EPO had lower inbreeding coefficients than WPO (Table 2.6).

Table 2.4. Numbers and percentages of inbred and outbred within- and extra-pair offspring. Figures for inbred and outbred offspring if all females were always faithful to their social partners, i.e. with no extra-pair offspring present in the population ("if faithful total") are also given for comparison. Percentages are presented per row and rounded to 1 decimal place. Any individual with inbreeding coefficient $f > 0$ was classified as inbred.

	inbred	outbred	row total
within-pair	130 7.5%	1597 92.5%	1727
extra-pair	115 4.3%	2589 95.8%	2704
observed total	245 5.5%	4186 94.5%	4431
if faithful total	459 10.4%	3972 89.6%	4431

Table 2.5. Effects of within-pair status of an offspring (whether it was within-pair, WP, or extra-pair, EP) on the offsprings inbreeding status (whether it was inbred, with $f > 0$, or outbred, with $f = 0$), using a binomial mixed model run in *MCMCglmm*. The model was run without mother-son pairings.

	inbreeding status of an individual	
	(inbred *vs* outbred)	
Fixed effects	**Posterior mean (95% CI)**	**P**
intercept	-9.83 (-10.80, -8.91)	<**0.001**
within-pair status (EP, WP)		
WP	1.36 (0.70, 1.98)	<**0.001**
Random effects	**Posterior mean (95% CI)**	
nest ID	25.12 (21.11, 29.51)	
cohort	2.55 (0.46, 5.23)	
Sample size	4283	

Table 2.6. Effects of within-pair status of an inbred (i.e. $f > 0$) offspring (whether it was within-pair, WP, or extra-pair, EP) on the offsprings level of inbreeding f (continuous), using a linear mixed model run in *MCMCglmm*. The model was run without mother-son pairings.

	inbreeding level of an individual	
	($0 < f \leq 0.125$)	
Fixed effects	**Posterior mean (95% CI)**	**P**
intercept	-4.99 (-5.70, -4.26)	<**0.001**
within-pair status (EP, WP)		
WP	0.50 (0.17, 0.77)	<**0.001**
Random effects	**Posterior mean (95% CI)**	
nest ID	4.02 (2.86, 5.17)	
cohort	1.63 (0.29, 3.32)	
residual variance	0.15 (0.11, 0.18)	
Sample size	245	

2.4. Discussion

My study explored the associations between, and the effects of, inbreeding, kinship, and infidelity in a wild population of a cooperatively-breeding bird. I found some support for each of the three key assumptions outlined in the Introduction as necessary for the hypothesis that extra-pair reproduction is driven by inbreeding-avoidance. Thus I found **(1)** evidence of inbreeding depression in nestling mass; **(2)** that increased kinship between social partners was associated with a higher frequency of extra-pair offspring in the brood; and **(3)** that extra-pair offspring were less likely to be inbred than within-pair offspring, and when inbred, they had lower inbreeding coefficients than within-pair offspring. However, detailed analysis revealed that kinship-infidelity results were context-specific: the frequency of extra-pair offspring only increased with kinship when considering mother-son pairs. Given that only 4.2% of broods had mother-son pairs as social parents, my results suggest that the inbreeding-avoidance hypothesis cannot explain the widespread occurrence of extra-pair reproduction in this system. I also found no evidence that any of these aspects were affected by the social environment. I discuss each of these points in turn below.

2.4.1. Inbreeding and inbreeding depression

Both social pairing between close relatives and moderate-level inbreeding were rare in my population: only 4.9% of pairs had $k_{soc} \geq 0.125$ and only 0.3% of individuals had f between 0.125 and 0.25 (Table 2.1). There was no high-level inbreeding ($f \geq 0.25$). The *overall* frequency of incestuous pairings and of inbreeding events were 10.5% ($k_{soc} > 0$) and 5.5% ($f > 0$) respectively. These overall frequencies increased with tighter restrictions on the pedigree to 24.6% incestuous pairs and 18.2% inbred offspring for 3+ known grandparents, and to 28.5% incestuous pairs and 21.5% inbred offspring with four grandparents known (Table A.1). The increased frequency presumably reflects, in part, the exclusion of pairs/individuals erroneously assigned $k_{soc} = 0$ and $f = 0$ because their ancestry information was not sufficient to identify lower levels of relatedness/inbreeding: without a perfectly complete pedigree, inbreeding between distant relatives may not be detected, leading to an underestimation of the overall occurrence of inbreeding. However, the steep increase beyond 2+ known grandparents is likely predominantly due to biasing the dataset towards females who have dispersed shorter distances from their natal territory, as the ability to sample all grandparents was often restricted to these females. These short-dispersing females will be more likely to encounter male relatives as partners than females dispersing over longer distances (in superb fairy-wrens female dispersal is obligatory [Mulder, 1995]). My results therefore indicate that inbreeding and social pairing between close relatives were rare in this population. The overall levels detected were nevertheless comparable to several other bird populations (reviewed in Kruuk *et al.* [2002a]). For instance, in a British great tit population, only 1.3% of pairings were between first- or second-order relatives [Szulkin *et al.*, 2007], whereas in my superb fairy-wren population it was 4.9% of

pairings (high/moderate k_{soc}, Table 2.1a). In contrast, the Mandarte Island song sparrows have a substantially higher frequency of closely-related pairings: 21.4% of pairings were between first- or second-order relatives [Reid *et al.*, 2015c].

I note also that theory suggests that under certain conditions father-daughter pairings may be more likely than mother-son pairings [Waser *et al.*, 1986]. However, in superb fairy-wrens, obligate female dispersal means that females never pair with their social fathers and so the only feasible social pairing of close/familiar relatives in this system are mother-son pairings (all of $k_{soc} = 0.25$ cases here). While a female could conceivably disperse and pair/mate with her extra-group sire, this has never been observed. Such pairings may be unlikely in this system due to the dispersal distances typically being too large to facilitate father-daughter contact. As a result, I do not see offspring resulting from pairings between females and their fathers.

I found evidence for inbreeding depression in nestling mass, of magnitude comparable to other studies (e.g. Soay sheep on the islands of St. Kilda [Berenos *et al.*, 2016]). However, although nestling mass positively affected survival, there was no evidence for inbreeding depression in survival. Inbred offspring with $f = 0.125$ surviving to fledging were on average 6.5% lighter than outbred offspring ($f = 0.125$: mean observed nestling mass ~6.6g; $f = 0$: mean observed nestling mass ~7.0g). The average survival of fledglings with nestling mass 6.5-6.7g was 54%, while for fledglings with nestling mass 6.9-7.1g it was 58%. Therefore, all else being equal, a reduction in nestling mass should have translated into ~4% reduction in survival for inbred fledglings ($f = 0.125$), but this was not evident from my survival models (in which estimates suggested inbreeding depression in survival, but 95% CIs spanned 0). Due to the rarity of inbreeding events between close relatives, the lack of statistical support for inbreeding depression in survival may be due, in part, to a lack of statistical power, but these calculations suggest that any reduction in survival within this period may not be large. I did not test for inbreeding depression in adult traits due to the low number of inbred adult birds. Thus detecting inbreeding depression may be easier in populations with higher levels of inbreeding. It may also be facilitated by use of high-density genomic marker data, which can reveal variation in genome-wide heterozygosity amongst individuals classified as $f = 0$ with a pedigree analysis [Huisman *et al.*, 2016]. Regardless, my analyses here do not indicate strong inbreeding depression in survival in this population.

Two further caveats are worth pointing out with regard to interpretation of the estimated occurrence of inbreeding depression. Firstly, it is of course difficult to ascertain how the level of inbreeding depression observed during the period of this study may compare to the severity of inbreeding depression in the past, and thus difficult to infer past selection pressures against inbred individuals. Second, whilst inbreeding depression of some form is necessary for inbreeding avoidance to provide a plausible explanation for extra-pair paternity, it would need to be sufficiently strong for the adaptive benefits of inbreeding avoidance to counter any costs and any potential inclusive fitness benefits of inbreeding [Szulkin *et al.*, 2013; Duthie and Reid, 2016; Duthie *et al.*, 2016a].

2.4.2. Infidelity variation with kinship

My analyses confirmed Cockburn *et al.*'s [2003] results: mother-son pairings resulted in absolute infidelity by the female, with no WPO produced by such pairings. These results appear robust to 'selective disappearance' as I found no evidence for reduced clutch size and/or survival to measurement age, as would happen if EPO were more likely to survive to measurement than WPO due to inbreeding depression [Reid *et al.*, 2015b]. However, there was no evidence of inbreeding avoidance through extra-pair reproduction in cases other than the mother-son pairs, who were the social parents of 4.2% of the observed broods. This, together with behavioural data indicating that all females seek extra-pair copulations throughout the breeding season [Cockburn *et al.*, 2016], suggests that the main reason for extra-pair reproduction for the majority of the females in this study is *not* inbreeding avoidance.

The lack of a simple population-wide relationship between k_{soc} and infidelity indicates that explanations for infidelity can be context-dependent. Brouwer *et al.* [2017] recently showed that explanations for infidelity patterns in the fairy-wren family vary depending on the level of analysis and the spatiotemporal scale used. In my study, only females in mother-son pairings avoided inbreeding through increased infidelity, and the hypothesis of inbreeding avoidance through EP reproduction was not supported for other females. Yet, studies typically investigate this hypothesis at a population-level, often assuming that a simple relationship, or lack thereof, between kinship and infidelity is evidence for the existence of a population-wide explanation for infidelity. Even across other species of Maluridae, several studies have shown higher infidelity in incestuous pairs [Brooker *et al.*, 1990; Tarvin *et al.*, 2005; Brouwer *et al.*, 2011; Varian-Ramos and Webster, 2012; Kingma *et al.*, 2013], but I note that where relatedness between social partners is assessed by genetic markers, it is not possible to distinguish certain types of relationships, such as mother-son pairings, for which different strategies may apply. Thus I suggest that care is needed when interpreting *overall* relationships between kinship and infidelity as evidence of a *general* inbreeding avoidance "strategy" applicable to all individuals in a population. My data suggest that such relationships may vary and that very different patterns in a relatively small number of individuals, not necessarily representative of the whole population, could drive the results of an analysis.

Furthermore, evidence suggests that, in general, bird species can only recognise kin when there is strong contextual evidence of relationship, such as having been raised in the same nest [Nakagawa and Waas, 2004; Ihle and Forstmeier, 2013]. It would seem unlikely that females are able to distinguish kin, beyond close/familiar relatives, from non-kin, and thus unlikely that they would actively allocate paternity based on kinship to a less-closely related social partner. Close relatives, such as nuclear family members and familiar individuals (e.g. a female's offspring, nest mates), may present a special case that should potentially be considered separately from more distant relatives in these types of studies.

2.4.3. Infidelity reduces inbreeding

The levels of inbreeding I detected were low: 94.3% of the inbred individuals had inbreeding levels of $f < 0.125$. Yet, I found that EPO were more likely to be outbred than WPO, even after the exclusion of the mother-son pairings, and that amongst inbred offspring, EPO had lower inbreeding coefficients than WPO. These results demonstrate that differences in inbreeding status and inbreeding levels between WPO and EPO can arise even without a relationship between kinship (k_{soc}) and infidelity (I found none when mother-son pairings were excluded).

Due to the lack of a relationship between kinship and infidelity beyond the mother-son pairings, active mate choice for less-related males seems unlikely to explain the difference in inbreeding status between WPO and EPO. What is driving this relationship? I suggest it is likely to be linked to the demographic and/or spatial structure of the population: females may be, on average, less closely related to males in another group, several territories away, than they are to their social partners. Potentially, this pattern could be linked to non-random formation and/or persistence of more-related - than expected by chance - social pairs [Reid *et al.*, 2015c], kin structure i.e. relatedness between individuals varying with distance [Nakagawa and Waas, 2004; Foerster *et al.*, 2006; Lee *et al.*, 2010; Leedale *et al.*, 2018], or constraints on mate availability [Duthie *et al.*, 2016a]. A more detailed analysis will be required to understand whether the spatial and/or temporal distribution of mates can explain the pattern I observed.

2.4.4. Assessment of the inbreeding avoidance explanation for extra-pair paternity

At first glance all three assumptions necessary for the hypothesis of inbreeding avoidance through EP reproduction - inbreeding depression, increase in EPP when social partners are related, and a reduction in inbreeding *via* infidelity - were met in my study system. However, dissecting the infidelity-kinship relationship I found that there was no population-wide pattern, and that the effect of kinship on infidelity was driven entirely by mother-son pairs. Looking beyond such pairings, the risk of mating with relatives appeared too low and the degree of kin recognition required too fine-scale for inbreeding avoidance to serve as a plausible explanation for EPP in this system. Given that only 0.7% of pairings involved moderate kinship levels ($0.125 \leq k_{soc} < 0.25$), i.e. at a level which could be relevant to female choice, it seems highly unlikely that a complex behaviour such as extra-pair reproduction would occur in the vast majority of breeding events just to mitigate this small risk of inbreeding: behavioural evidence suggests that females always seek EP copulations *via* extra-territorial forays [Cockburn *et al.*, 2016], and 82.8% of broods have at least one EP offspring. It therefore seems likely that other explanations for extra-pair reproduction, such as choice of a preferred male in light of restricted social partner choice (e.g. choosing males with earlier moult date [Dunn and Cockburn, 1999; Cockburn *et al.*, 2008a]), are more plausible. My study therefore illustrates the need to test all components of the hypothesis of inbreeding avoidance through EP reproduction explicitly:

specifically, relationships between kinship and infidelity, as well as differences between WPO and EPO, need to be assessed in detail.

2.4.5. Social environment

The social environment influenced patterns of infidelity, but not the occurrence or consequences of inbreeding. It had a strong influence on infidelity rates, with females assisted by helpers producing more EPO, predominantly sired by males outside of the entire social group (95% of the EPO were extra-group offspring; Chapter 3); in particular, for reasons that are not entirely clear, the number of unrelated helpers is the best predictor of levels of extra-group paternity (Chapter 3). However, the presence of helpers did not affect the probability of offspring in a nest being inbred (Table A.8). Furthermore, whilst nestlings from broods assisted by helpers were heavier than those from unassisted broods (as in Kruuk *et al.* [2015]), there was no evidence that the presence of helpers affected the occurrence or magnitude of inbreeding depression (Table 2.2; Table A.3). These results fit with the general impression that it may be difficult to detect interactions of inbreeding depression with environmental conditions in natural populations [Pemberton *et al.*, 2017]. However, it is possible that I was simply unable to find support for interactions with inbreeding depression in my study population due to the rarity of inbreeding events.

2.4.6. Conclusions

My study used multigenerational pedigree data from a long-term individual-based study of a wild population to investigate patterns of inbreeding and inbreeding depression, as well as causes and consequences of extra-pair reproduction. I showed that the relationship between kinship and infidelity may be complex, context-dependent and not necessarily population-wide, and that results can be easily driven by a small sample of individuals - in this case mother-son pairings. Additionally, I showed that within-pair and extra-pair offspring can differ in their probability of being inbred, even when infidelity occurs for reasons apparently unrelated to inbreeding avoidance. Furthermore, the social environment affected infidelity rates, but did not affect the probability of offspring being inbred or inbreeding depression. Overall, my study demonstrates how the social system of a population may affect mating patterns and their consequences in multiple complex ways, and also illustrates the value of long-term pedigree data for providing insights into core aspects of evolutionary biology.

Chapter 3

Unexpected effects of helper relatedness on female infidelity in a cooperative breeder

ABSTRACT

Phenotypes of individuals are frequently affected by the conspecifics with whom they interact. This may be particularly important for cooperative breeders, in which breeding pairs are assisted by helpers of varied relatedness. While the presence of helpers may affect mating patterns and infidelity levels in such systems, the effect that helpers of different relatedness have on the breeding pair is not clear. I explored the effects of helper-relatedness on female infidelity in a cooperatively-breeding passerine. I used a long-term study of superb fairy-wrens (*Malurus cyaneus*), in which groups consist of a dominant pair and male helpers who may or may not be sons of the breeding female. The presence of helper-sons did not affect the frequency of extra-pair paternity (57% for females with no helpers, 58% for females with only helper-sons). However, the presence of unrelated helpers was associated with increased rates of both extra-pair paternity and, unexpectedly, of extra-group paternity. Broods produced in groups assisted by at least one unrelated helper contained 67% extra-group offspring and 77% extra-pair offspring, while those in groups assisted solely by helper-sons contained 58% extra-group offspring. I found no effects of helper-relatedness on nest productivity. Our results illustrate how relatedness between individuals can influence conspecific interactions in complex ways, and that infidelity in this highly unfaithful species cannot be explained by the 'constrained-female' hypothesis.

3.1. Introduction

Animals rarely live in isolation: to varying degrees, they are surrounded by conspecifics with whom they interact. Since interacting individuals can affect each other's phenotypes, this 'social environment' can influence multiple aspects of an individual's life history and hence its fitness. Such interactions will be especially important in group-living species, and will vary depending on characteristics of the interacting individuals - for example, the relatedness between them. In cooperatively-breeding species, breeding pairs are often assisted by 'helpers' related to the dominant pair [Green *et al.*, 2016], and the presence of these helpers can have implications for offspring care and group productivity. Moreover, the presence of helpers may also affect mating patterns. Females of many cooperative breeders exploit the presence of additional males in the group by mating with them. By contrast, although the presence of helpers has sometimes been implicated in rates of extra-group paternity (Chapter 2), in general the influence of helper presence on infidelity outside of the social group has been under-explored. For instance, is helper relatedness relevant for infidelity? Whilst there is evidence from cooperatively-breeding species that the relatedness of helpers to the breeders within a group may affect offspring care [Green *et al.*, 2016], there have been few tests of the extent to which the relatedness of helpers in an individual's social environment affects overall mate choice patterns.

In cooperatively breeding species, helpers may influence several aspects of how a breeding pair

lives. They can help the dominants by provisioning young and defending the brood and territory from predators and conspecific intruders. This can either improve reproductive success or allow the breeders to reduce their own workload (load-lightening), potentially improving their survival (for recent examples see Meade *et al.* [2010]; Dixit *et al.* [2017]; Guindre-Parker and Rubenstein [2018]). While provisioning of young is inarguably the core role of helpers, helpers may assist with a wide range of activities and contribute to nest productivity in many ways, such as for example nest defence [Austad and Rabenold, 1985; Mumme, 1992] or even thermoregulation [Arnold [1990]; Du Plessis [2004] p. 120]. Furthermore, the effort and impact of helpers may vary with several factors, including the relatedness of helpers to other members of the group. Most helping occurs within kin groups [Dickinson and Hatchwell, 2004] and decisions regarding whether to help or not, or how much to help, may be based on the helper's relatedness to the breeding pair. For example, in long-tailed tits, individuals will only become helpers if they can assist relatives [Russell and Hatchwell, 2001]. Moreover, even after the decision to help has been made, helpers may adjust their helping behaviour based on relatedness: a comparative analysis of 36 cooperatively-breeding bird species showed that, although there is substantial variation, in most species helpers more closely related to the offspring provide more care than do unrelated helpers [Green *et al.*, 2016]. In meerkats, recently pregnant and pregnant females are more likely to allo-lactate if they are related to the litter mother [MacLeod *et al.*, 2013], although 'babysitting' contributions of helpers are not associated with their relatedness to the pups [Clutton-Brock *et al.*, 2000]. Thus relatedness can clearly play a major role in shaping within-group dynamics. However, the role of helper relatedness in other aspects of group life, such as its effects on mating patterns of breeding individuals, is less well explored.

In this study, I investigated the associations between infidelity and helper relatedness to the breeding female in a cooperative breeder with high infidelity rates, the superb fairy-wren (*Malurus cyaneus*). The Maluridae (fairy-wrens) are socially monogamous cooperatively breeding birds, for which the presence and number of helpers is known to affect female infidelity, with rates of extra-pair paternity (EPP) typically increasing in the presence of helpers in a group [Mulder *et al.*, 1994; Brouwer *et al.*, 2017] (Chapter 2). One explanation for this association has been the 'constrained female' hypothesis (CFH): female fidelity may be driven by the need for paternal care of offspring, but in the presence of helpers, this 'constraint' is lifted and a female can afford the risk of losing paternal care from her cuckolded social partner [Mulder *et al.*, 1994; Gowaty, 1996, 1997]. Females assisted by helpers and provided with an alternative or additional source of offspring care should therefore be more likely to engage in extra-pair reproduction. Moreover, if the dominant male has to 'keep track' of all the subordinate males on his territory as well as the female, then with an increase in the number of helpers in a group, he may have less control over his partner, making infidelity easier for her [Mulder *et al.*, 1994; Cohas *et al.*, 2006]. However, detailed investigation into the role of helpers in shaping mating patterns has been limited to date. Thus despite these associations between helpers and infidelity, and the importance of within-group relatedness for dynamics of cooperatively-breeding groups, the importance of helper relatedness for female infidelity is unclear.

In this socially monogamous species, breeding pairs may be assisted by up to four (very rarely five) male helpers, though unassisted pairs are common. These helpers are behaviourally subordinate to the dominant male, but reproductively mature: they can sire young and their testosterone levels are no different from those of dominant males living in pairs [Peters *et al.*, 2001]. The dominant males living in groups with helpers show higher levels of testosterone than other males [Peters *et al.*, 2001], presumably as they need to assert their dominance; dominants punish helpers if they are experimentally prevented from caring for offspring [Mulder and Langmore, 1993]. Helpers provision young and help to defend the territory; their presence is associated with increased nestling mass [Kruuk *et al.*, 2015] (Chapter 2) and there is evidence that the presence of helpers affects female investment in eggs [Russell *et al.*, 2007; Langmore *et al.*, 2016]. Infidelity rates are very high in the superb fairy-wrens [Mulder *et al.*, 1994; Cockburn *et al.*, 2016], and increase further in the presence of helpers (Chapter 2). Superb fairy-wren helpers vary in their relatedness to the female on the territory, with 62% of helpers being the breeding female's sons from a previous brood and 38% not her sons; previous work shows that mothers never breed with their sons [Cockburn *et al.*, 2003] (Chapter 2)[1].

Here I used long-term data from a population of superb fairy-wrens in south-eastern Australia to investigate the effect of the social environment on mate choice using a mixed-modelling approach. I first assessed rates of extra-pair paternity in detail, partitioning the extra-pair paternity into within-group extra-pair (i.e. paternity going to helpers in the group) *vs* extra-group paternity (i.e. involving a male in a different group; see Box 1 for definitions). I then tested whether the relatedness of helpers to the breeding female affected her extra-pair mating behaviour and the distribution of paternity in the population by categorising helpers either as sons of the breeding female or unrelated helpers. Because the 'constrained female' hypothesis relies on helpers' contributions to nestling care, I then also tested for differences between helpers of different relatedness in nest productivity. Previous work in this population did not find any indication of differences between related *vs* unrelated helpers in provisioning rates (Dunn *et al.* [1995], A. Cockburn, unpubl.), but to assess whether there were any effects of helper relatedness on nest productivity, I tested for associations between the numbers of helper-sons or of unrelated helpers and nestling performance.

3.2. Methods

3.2.1. Study system

The study population consists of a colour-banded population of superb fairy-wrens living in a ~60 ha area located in and around the Australian National Botanic Gardens, Canberra, Australia (35°16 S, 149°06 E). Each year since 1988 up to 90 territories have been monitored. Data used

[1]Since I carried out this analysis, individuals hatched after 2013 were genotyped using SNP data, providing the first evidence of nuclear family inbreeding event in this population.

in this study span 26 years (1988-2013). The study population is censused all 52 weeks of the year [Cockburn *et al.*, 2003], including details of the presence and identity of helpers on each territory. During the breeding season progress of all nests is monitored, with nestlings banded 5-8 days post-hatching, when blood samples are taken for genotyping [Double *et al.*, 1997b] (Chapter 2). Up to eight clutches can be started by each female and up to three broods raised to fledging in a single season, with 1-4 offspring in each brood and a modal clutch size of three [Cockburn *et al.*, 2008c].

Superb fairy-wrens have very high rates of extra-pair and extra-group paternity: ~83% of broods have at least one extra-pair offspring (Chapter 2) and the majority of these are also extra-group (see Table 3.1 below). Here I categorised offspring as to whether they were the result of within-pair, within-group extra-pair, or extra-group paternity (see Box 1 for terminology). In the analyses below, I compared rates of (a) extra-pair *vs* within-pair paternity; and (b) extra-group *vs* within-group paternity.

Second, I categorised helpers based on their relatedness to the breeding female, splitting them into two groups: sons of the breeding female ('helper-sons') *vs* others (which I call 'unrelated helpers'). I chose this simple distinction of 'helper-sons' *vs* 'unrelated helpers', rather than using any finer-scale relatedness between individuals, as my previous work investigating the associations between the kinship of the social partners and infidelity showed that while females do not mate with their sons, infidelity levels do not vary with more distant relatedness (Chapter 2). These results suggest that females are not able to distinguish finer levels of relatedness, and I therefore combined these into the single category which I refer to as 'unrelated helpers'.

BOX 1: TYPES OF PATERNITY

- **within-group (WG):** offspring sired either by the social partner or helpers within the focal social group [WG = WP + WGEP]
- **within-pair (WP):** offspring sired by the breeding female's social partner, the dominant male on the focal territory
- **within-group extra-pair (WGEP):** offspring sired by a helper within the focal social group
- **extra-pair (EP):** offspring sired by any male other than the breeding female's social partner (either within- or extra-group) [EP = WGEP + EG]
- **extra-group (EG):** offspring sired by a male from outside the social group that reared the offspring

Social pairings between mothers and sons occur in ~4% of all breeding events. These pairings

result in 100% female infidelity (all offspring are both EP and EG [Cockburn *et al.*, 2003]; Chapter 2) and furthermore almost always (except for one brood in my dataset) all helpers assisting mother-son pairs are also sons of the breeding female. These mother-son pairings appear to be special cases in which mating patterns are driven by inbreeding avoidance (Chapter 2), and therefore, for clarity, I excluded them from the current analyses.

3.2.2. Statistical analyses

All analyses were carried out in *R* version 3.3.1 [Development Core Team, 2011] using two packages: *ASReml-R* version 3 [Butler *et al.*, 2009] for response variables with Gaussian errors, and *MCMCglmm* [Hadfield, 2010] for binomial errors. Significance of the fixed effects was assessed using Wald statistics for models run in *ASReml-R*, while in *MCMCglmm* terms were considered statistically significant when the 95% credible intervals (CIs) did not span zero and the *pMCMC* values were < 0.05. In *MCMCglmm* models I also aimed for effective sample size of 2000 and autocorrelation below 0.1, ensuring that the resulting effective sample sizes are above 1000. For iteration/prior information for *MCMCglmm* models see SI.

3.2.3. Effects of helper relatedness on female infidelity

I fitted binomial generalised linear mixed effects models using the *R* package *MCMCglmm*. Our models separately considered: (a) the `proportion of extra-pair offspring` in a brood (EG and WGEP); and (b) the `proportion of extra-group offspring` in a brood (just EG). In both models the fixed and random structures were the same. **Fixed Effects:** For each brood, the `number of helper-sons` (0-4) and `number of unrelated helpers` (0-4) were fitted as the main variables of interest. I also fitted `mother's age` and the age of the dominant male on the territory (`social father's age`), using two-level factors of one-year-old *vs* older, as one-year olds are attending their first broods, while older birds have prior experience with rearing young. **Random Effects:** `mother ID` and `social father ID` were fitted to account for multiple observations; `cohort` was fitted as a multilevel factor to account for year-to-year variation (1988-2013; each cohort consists of nestlings from one breeding season, i.e. from August to March).

I additionally re-ran the above models on randomly-selected subsets of 50% of the dataset ten times, to carry out within-study replication (see SI for details).

3.2.4. Effects of helper relatedness on nest productivity

To assess whether the presence of helpers of different levels of relatedness had implications for nest productivity, I assessed offspring performance by modelling (i) offspring survival to measurement age (5-8 days after hatching), and (ii) nestling mass.

(i) Early survival

I fitted a generalised linear mixed effects model in *MCMCglmm*, with binomial errors and a response variable of the `proportion of offspring in a brood surviving to measurement age` (with the original brood size defined as equal to the number of eggs and the number surviving defined as the number of nestlings 5-8 days after hatching). **Fixed Effects:** I fitted fixed effects of the `number of helper-sons` (0-4), `number of unrelated helpers` (0-4), `mother's age` and `social father's age` (one-year-old *vs* older), and the average `nestling age` at banding for each brood. **Random Effects:** I fitted `mother ID`, `social father ID` and `cohort` as random effects, as above.

(ii) Nestling mass

I fitted a linear mixed effect model in *ASReml-R*, with nestling mass as the response variable, and Gaussian errors. **Fixed Effects:** I fitted the `number of helper-sons` (0-4) and `number of unrelated helpers` (0-4) to test for associations between helper-relatedness levels and nestling mass. The `inbreeding coefficient` of each offspring was also fitted to account for inbreeding depression in nestling mass (Chapter 2). I also fitted `brood size` (as a covariate) to account for differences in the competition for resources provided to each offspring in broods of different size. Additionally, `sex` of the nestling (female *vs* male) was fitted to account for size differences between the sexes. I fitted `nestling age` at measurement in days to account for the fact that nestlings were weighed at different ages (days 5-8); a quadratic term for `nestling age` was fitted because nestlings can be approaching adult weight by day 8, and a two level factor '`1992`' (pre-1992 *vs* 1992+) to account for a weighing protocol change in 1992 [Kruuk *et al.*, 2015]. **Random Effects:** I fitted `nest ID` to account for similarities between siblings from the same brood; a multi-level factor of `hatch date` (twelve two-week intervals, between 23 September and 15 March) to account for intra-annual variation; and `cohort` as above.

Given the results of the nestling mass model (see below), I also fitted an alternative formulation of the model with the total number of helpers of both types as a three-level factor (0, 1 and 2+) and 'group type' as a three-level factor to represent groups of either all helper-sons, all unrelated helpers or mixed relatedness (i.e. both types of helper). These terms replaced the fixed effects of the numbers of unrelated helpers and helper-sons in the above models, but the rest of the fixed and random effects were identical to those described above. See the SI for details.

Finally, I investigated whether the relatedness of the helpers to the mother affected her investment into the breeding attempt (see SI for details).

3.3. Results

3.3.1. Effects of helper relatedness on female infidelity

The dominant pair on the territory was assisted by one or more helpers in 879 of 1936 broods (45%). Of these, 363 were assisted exclusively by helper-sons, 435 were assisted exclusively by unrelated helpers, and 81 were assisted by a mix of unrelated helpers and helper-sons.

Infidelity levels were very high: across all broods, 62% of offspring were a product of infidelity - Table 3.1 shows the proportions of within-pair, within-group extra-pair and extra-group paternity for the broods of different types. Broods with no helpers and broods with only helper sons had no within-group extra-pair paternity (WGEP; Table 3.1); females do not mate with their sons. There was no indication of any difference in the proportion of extra-group (EG) offspring between the unassisted groups and groups supported by helper-sons (Table 3.1). However, groups assisted by unrelated helpers had a higher proportion of EG offspring (an average of 67% in groups with at least one unrelated helper; Table 3.1) than groups without any unrelated helpers (an average of 58%; Table 3.1 - helper-sons).

The results of the GLMMs of proportions of extra-pair and extra-group paternity are given in Table 3.2. The proportion of both extra-pair (Table 3.2a) and extra-group (Table 3.2b) offspring in the brood increased with the number of unrelated helpers, but not with the number of helper-sons (Figure 3.1, Table 3.2). Note that on Figure 3.1 the "zero" category represents broods where there are *no helpers of that particular type* - which includes both broods that are completely unassisted (and have the lowest infidelity rates at ~57%, Table 3.1) and broods that are assisted by the helpers of the *other* type - Figure 3.2 presents the information in an alternative way to aid visualisation. Mother's age increased infidelity: females older than one-year-old had a higher proportion of both EP and EG offspring. In contrast, older social fathers had a lower proportion of EG offspring than one-year-old social fathers (Table 3.2). Even when correcting for these age effects, there was substantial variance between females and also between dominant males ('social fathers') in the rates of both EP and EG paternity (Table 3.2, random effects). When I re-ran the models ten times on 50% subsets of the data, the results of the replication analyses confirmed the association between the presence of unrelated helpers and increase in EP and EG levels, but the associations between the mother and social father ages and infidelity were not always consistent across different subsets (Figure B.1).

Table 3.1. Mean proportions of within-pair (WP), within-group extra-pair (WGEP) and extra-group (EG) paternity across broods, split by the presence and relatedness type of helpers (helper-sons *vs* unrelated helpers) in the group. The values shown are the means of the proportions calculated for each brood (please note this is very similar to, though not exactly the same as, the proportion of individual nestlings of each type). The data-set contains unassisted broods, broods assisted exclusively by helper-sons, broods assisted exclusively by unrelated helpers, and broods assisted by a mix of unrelated helpers and helper-sons. These data are presented as follows: **(a)** all broods (assisted and unassisted); unassisted broods (no helpers); assisted broods (at least one helper of any relatedness type); **(b)** assisted broods, showing the effect of helper relatedness type (at least one helper-son *vs* at least one unrelated helper); **(c)** assisted broods, showing the effect of group composition with regard to helper relatedness type (only helper-sons *vs* only unrelated helpers *vs* a mix of helper-sons & unrelated helpers). Total n = 1936 broods.

	paternity proportion			
type of help	**WP**	**WGEP (extra-pair)**	**EG**	**sample size (n broods)**
(a) all broods				
all broods	0.377	0.027	0.596	1936
no helpers	0.431	0.000	0.569	1057
$\geq$ 1 helper	0.311	0.060	0.628	879
(b) assisted broods - effect of helper relatedness				
$\geq$ 1 helper-son	0.392	0.016	0.592	444
$\geq$ 1 unrelated	0.232	0.103	0.665	516
(c) assisted broods - effect of group composition				
helper-sons	0.425	0.000	0.575	363
mixed	0.245	0.085	0.670	81
unrelated	0.230	0.106	0.665	435

Table 3.2. GLMM of the influence of the numbers of helper-sons and unrelated helpers on the proportion of (a) extra-pair and (b) extra-group offspring in a brood. Analyses were carried out at the brood level in *MCMCglmm* for (a) numbers of extra-pair *vs* within-pair offspring; and (b) numbers of extra-group *vs* within-group offspring. Models were run in *MCMCglmm* with binomial errors and thus estimates and variance components reported are based on posterior means, with 95% credible intervals (CIs).

	proportion of offspring resulting from infidelity			
	(a) extra-pair offspring		(b) extra-group offspring	
Fixed effects	**Estimate (95% CI)**	**P**	**Estimate (95% CI)**	**P**
intercept	0.37 (0.03, 0.72)	**0.038**	0.43 (0.06, 0.75)	**0.014**
mother age (1yo, older)				
older	0.39 (0.11, 0.64)	**0.001**	0.40 (0.14, 0.66)	**0.004**
social father age (1yo, older)				
older	-0.29 (-0.65, 0.03)	0.107	-0.38 (-0.72, -0.04)	**0.030**
number of helper-sons	0.09 (-0.08, 0.26)	0.304	0.10 (-0.07, 0.27)	0.272
number of unrelated helpers	1.02 (0.84, 1.22)	<**0.001**	0.47 (0.29, 0.65)	<**0.001**
Random effects	**Variance (95% CI)**		**Variance (95% CI)**	
mother ID	0.54 (0.21, 0.84)		0.58 (0.27, 0.90)	
social father ID	0.60 (0.30, 0.92)		0.54 (0.25, 0.83)	
cohort	0.01 (1.34^{-8}, 0.05)		0.02 (1.62^{-8}, 0.06)	
residual variance	1.75 (1.30, 2.17)		1.80 (1.37, 2.23)	
Sample size	1929		1929	

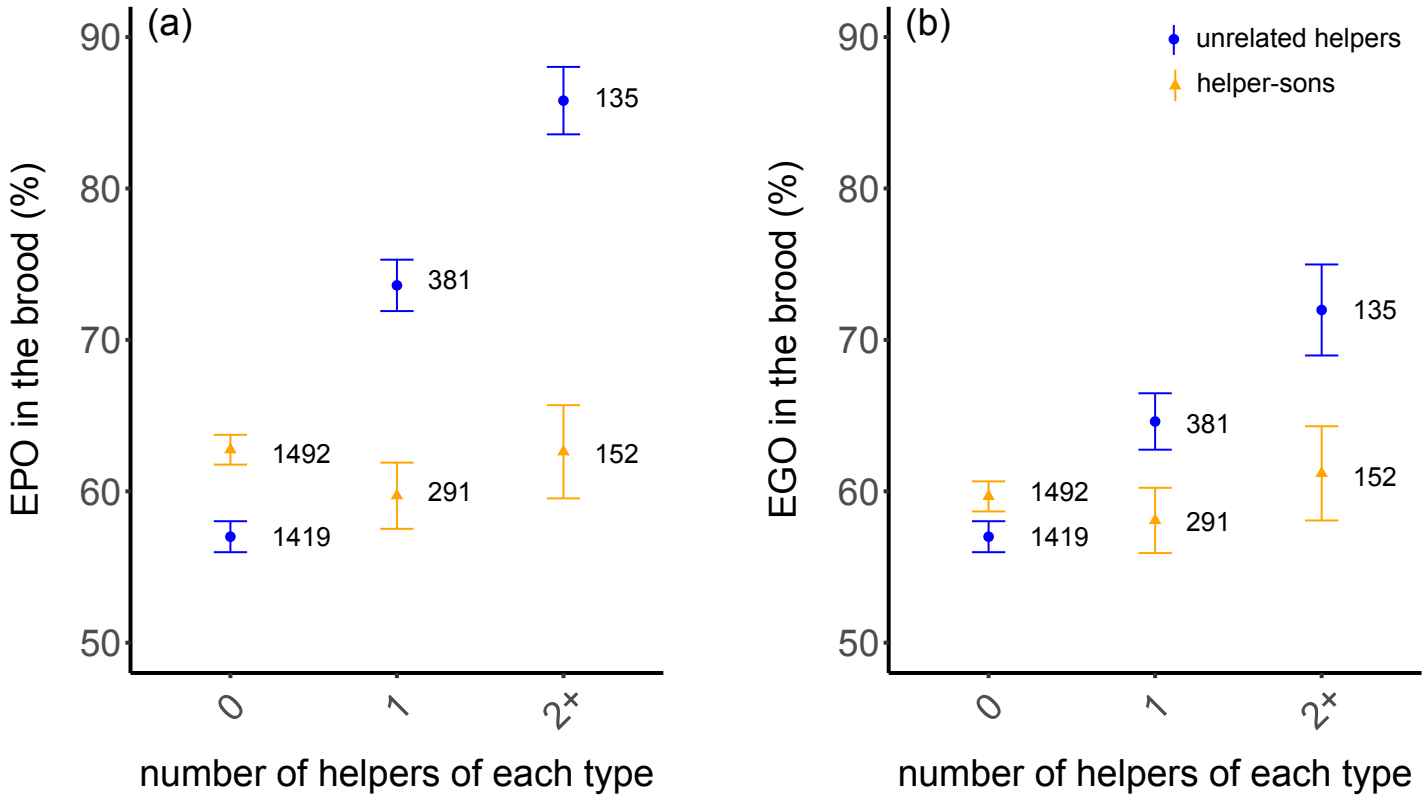

Figure 3.1. Effects of the numbers of helper-sons (orange triangles) and unrelated helpers (blue dots) on percentages of (a) extra-pair offspring (EPO), and (b) extra-group offspring (EGO) in a brood. Points represent means for EPO or EGO percentages in each brood (raw data for all broods) per helper category; there were few broods with 3 or 4 helpers of either type; to aid visualisation I binned these with broods with 2 helpers to create the 2+ category. Error bars represent $\pm$ 1 standard error for the binned raw data. Sample sizes for helper-sons and unrelated helpers for each bin are given next to the points. Note that the y-axes start at 50% - infidelity was ubiquitous in this population.

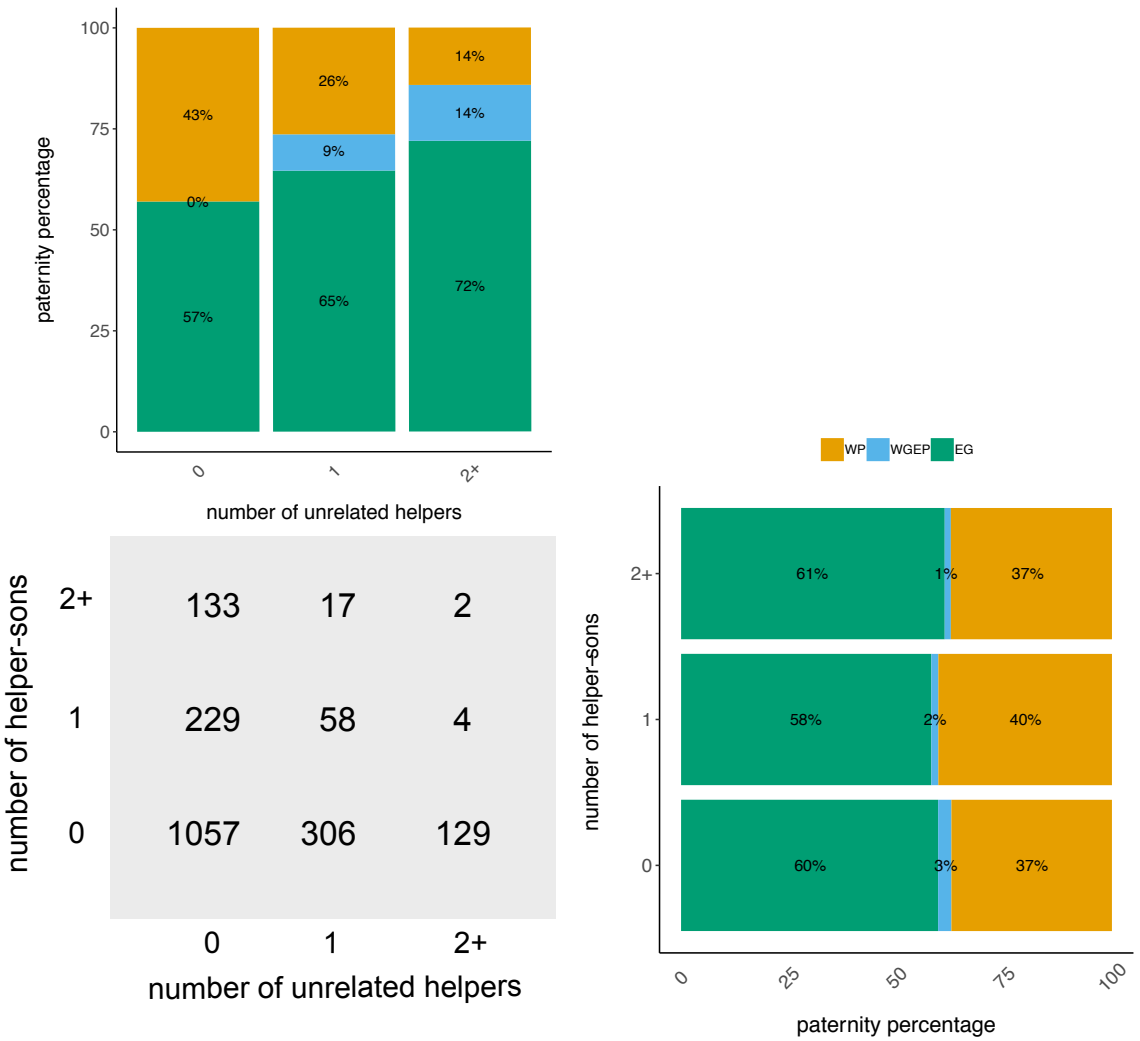

Figure 3.2. Matrix illustrating the numbers of unrelated helpers and helper-sons per brood, with bar charts illustrating the percentage of within-pair (WP), within-group extra-pair (WGEP), and extra-group (EG) per each category. Note: (i) that the low levels of WGEP in the right-hand panel will be going to unrelated helpers in those groups; and (ii) in the top panel, WGEP = 0% in groups with no unrelated helpers because all WGEP is always *via* unrelated helpers. There were few broods with 3 or 4 helpers of either type, to aid visualisation I binned these broods together with 2 helpers to create the 2+ category.

3.3.2. Effects of helper relatedness on nest productivity

Increase in nestling mass was associated with the increase in numbers of both helper-sons and unrelated helpers, to almost exactly the same degree (Table 3.3). To further test for any potential impact of helper relatedness levels I ran an additional model, which also confirmed that it was the total helper number, rather than relatedness, that was associated with nestling mass (Table B.2). Inbred offspring and offspring from larger broods were lighter than outbred offspring and offspring from smaller broods. Male nestlings were heavier than females, and nestling mass increased with age at measurement (Table 3.3). There was no association between the numbers of helpers of different relatedness-type and survival to measurement age (Table 3.4; note that *MCMCglmm* constrains variance components to be positive, thus the low values for random effects suggest that none of the phenotypic variation can be attributed to these random effects).

Finally, my investigation into maternal investment showed no association between the effects of different levels of helper relatedness and clutch size (Table B.1).

Table 3.3. Association between the numbers of helper-sons/unrelated helpers and nestling mass (see also Table B.2). This analysis was carried out at the level of individual offspring, in *ASReml-R*. Standard errors (SE) for the estimates and variance components are reported in brackets.

	nestling mass	
Fixed effects	**Estimate (SE)**	**P**
intercept	-3.69 (1.13)	**0.002**
1992 (1992+, pre-1992)		<**0.001**
pre-1992	0.58 (0.10)	
nestling age	2.10 (0.33)	<**0.001**
nestling age^2	-0.08 (0.02)	<**0.001**
brood size	-0.05 (0.02)	**0.021**
sex (female, male)		<**0.001**
male	0.15 (0.02)	
inbreeding coefficient	-3.07 (1.18)	**0.010**
number of helper-sons	0.10 (0.02)	<**0.001**
number of unrelated helpers	0.10 (0.02)	<**0.001**
Random effects	**Variance (SE)**	
nest ID	0.26 (0.01)	
hatch date	0.01 (0.01)	
cohort	0.01 (0.01)	
residual variance	0.24 (0.01)	
Sample size	4910	

Table 3.4. Effects of the numbers of helper-sons and unrelated helpers on survival of offspring to measurement age (5-8 days post-hatching). These analyses were carried out at the brood level in *MCMCglmm*. Estimates and variance components reported are based on posterior means and 95% credible intervals.

	early survival	
Fixed effects	**Estimate (95% CI)**	**P**
intercept	-0.24 (-0.90, 0.36)	0.474
average nestling age	0.01 (-0.07, 0.10)	0.845
mother age (1yo, older)		
older	0.03 (-0.12, 0.17)	0.731
social father age (1yo, older)		
older	-0.01 (-0.20, 0.18)	0.939
number of helper-sons	0.01 (-0.08, 0.10)	0.814
number of unrelated helpers	0.01 (-0.09, 0.10)	0.928
Random effects	**Variance (95% CI)**	
mother ID	0.001 (2.35^{-10}, 0.004)	
social father ID	0.001 (2.13^{-10}, 0.004)	
cohort	0.001 (1.52^{-12}, 0.005)	
residual variance	n/a	
Sample size	1838	

3.4. Discussion

This study used a long-term dataset to investigate the associations between female infidelity and the relatedness of the breeding female to the male helpers on her territory. I found that rates of infidelity increased with the number of helpers on a territory, but only when the helpers were unrelated to the breeding female: for example, rates of extra-pair paternity were 77% when a female had at least one unrelated helper, but only 58% if she had only helper-sons (but no unrelated helpers) and 57% if she had no helpers. Below I discuss possible explanations for these results.

The constrained female hypothesis explanation for extra-pair paternity [Mulder *et al.*, 1994; Gowaty, 1996, 1997] proposes that female infidelity in socially monogamous species is more likely when resources are abundant, as the 'constraint' of the need for paternal care of the offspring is reduced. As a corollary, in cooperatively breeding systems, females assisted by helpers should have higher rates of infidelity than unassisted females. Superb fairy-wren females abandon the nest if the number of helpers they have available is suddenly reduced [Dunn and Cockburn, 1996], suggesting they are indeed constrained and thus a good candidate species to test this hypothesis.

However, in my study, infidelity increased when females were assisted by unrelated helpers, but not for females assisted by helper-sons. Thus the only way in which my results could be compatible with the CFH would be if helper-sons provided no help, but unrelated helpers did provide useful assistance. This differential care would mean that the 'constraint' on females was lifted with unrelated helpers, but not helper-sons. This is difficult to test directly, but I found no evidence of any associations between helper relatedness and any measures of nest productivity, indicating no differences in care levels. There is also no evidence in this population that helper-sons and unrelated helpers provision nestlings at different rates (Dunn *et al.* [1995]; A. Cockburn, unpubl.). If anything, evidence across other bird species suggests that closely-related helpers typically provide more care [Green *et al.*, 2016]. Thus my results appear to be at odds with the CFH as an explanation for extra-pair paternity in superb fairy-wrens: a female with only helper-sons was no more likely (58%) to cuckold her social partner than an unassisted female (57%).

Debate over explanations for the occurrence of extra-pair paternity is still ongoing [Eliassen and Jorgensen, 2014; Forstmeier *et al.*, 2014]. I have recently shown that the high levels of EPP in this population are unlikely to be driven by inbreeding avoidance except in the extreme case of mother-son pairings (Chapter 2). Given the incompatibility of my results with the constrained female hypothesis, the most plausible explanation for the occurrence of EPP is therefore that a female uses it to realise her optimum mate choice without the limitation of needing to be socially paired to him [Cockburn *et al.*, 2013] - but that somehow the distribution of paternity is differentially affected by (or at least associated with) the presence of unrelated helpers. Below, I consider how this might occur and propose future work.

One obvious explanation for increased extra-pair paternity in the presence of unrelated helpers is that these helpers sire offspring within their social groups. However, most extra-pair offspring were sired by males from *other* groups (extra-*group* paternity; Table 3.1). This was true regardless of helper presence, numbers and relatedness. Unrelated helpers secured little (3%) within-group paternity. However, the lack of within-group success is not a simple case of inability to sire offspring. Helpers are sexually mature [Mulder and Cockburn, 1993; Peters *et al.*, 2001] and fertile: they secure EG paternity relatively often [Double and Cockburn, 2003; Cockburn *et al.*, 2009]. Helpers are of lower social status and tend to be younger than the dominant males [Cockburn *et al.*, 2008b], which may explain, at least partially, their lower reproductive success. However, these characteristics alone could not explain the *increase* in the proportion of extra-*group* offspring in the broods assisted by unrelated helpers.

What then explains the unexpected contrast between an association of infidelity and the numbers of unrelated helpers, but not numbers of helper-sons? I suggest that this association could arise either indirectly due to the spatial structure of the population, or more directly through changes in the behaviour of the breeding female and/or the behaviour of the dominant male.

Firstly, spatial heterogeneity in environmental conditions may influence the rates of extra-pair reproduction. Population density is expected to increase with the quality of the habitat. Higher density is likely to result in higher encounter rate, which could translate into increased availability of potential extra-pair mates [Birkhead, 1978; Westneat and Sherman, 1997] and thus increased infidelity [Griffith *et al.*, 2002]. Therefore, if there are more helpers in the higher density areas than in sparsely populated areas, I could see an association between the number of helpers and infidelity rates. However, both the numbers of helper-sons and unrelated helpers could be increased in high-quality areas. For instance, higher-quality habitat could attract unrelated helpers from other areas. On the other hand, more productive pairs may produce more sons and thus may potentially have more helpers - leading to the increased frequency of helper-sons, rather than unrelated helpers. Additionally, since superb fairy-wren females may travel over relatively long distances (several territories [Double and Cockburn, 2000]) in order to mate extra-pair, it is not clear at which scale the habitat quality and/or population density should be considered. To what extent is the local density relevant to female infidelity if females do not mate locally? Without further spatial analyses I cannot attribute the association between female infidelity and the relatedness of helpers to potential variation in population density. It is also worth nothing that it is possible, even if unlikely in this system, that behavioural differences between females play a role; for instance that females that are more likely to move territories (and so end up with unrelated helpers) are also more likely to mate with extra-group males.

Secondly, the link between infidelity and helper relatedness could be more direct. Unrelated helpers could affect the behaviour of the breeding female and/or of the dominant male in ways that change the distribution of paternity. Given that females do not breed with their sons, dominant males do not compete with helper-sons for within-group matings. However, unrelated helpers can sire offspring with the breeding female. Therefore, dominant males surrounded by

unrelated helpers could be adjusting their behaviour in an attempt to reduce within-group extra-pair matings, and thus attempting to prevent access of potential extra-pair mates to the female through mate-guarding. While the dominant males often leave the territory throughout the day to display to other females [Green *et al.*, 1995], there is some indication of mate guarding: the dominant male may defend the female vigorously against helpers in the 30-minute window after she has returned from the extra-group foray (this is described most fully on p. 139 of Cockburn *et al.* [2016]) - this may even involve extreme violence. Behaviours crucial to extra-pair reproduction, such as pre-dawn female extra-group forays [Double and Cockburn, 2000] and 'hidden lekking' during the dawn chorus [Cockburn *et al.*, 2009] occur during the early mornings. While forays and lekking have been investigated, subtler changes in behaviour in response to helper relatedness, particularly at the time of the forays, would be more difficult to observe.

It has also been shown that dominant males in groups with helpers have elevated testosterone levels relative to those without helpers [Peters *et al.*, 2001], suggesting that the dominant males need to assert their dominance in the presence of helpers. However, at present we do not know whether the testosterone elevation occurs in the presence of any helpers or only in the presence of unrelated helpers. The dominant male may have limited information on the relatedness between the breeding female and the helpers in the group. However, it is feasible that they could be responding to changes in female behaviour, and using these to estimate cuckoldry risk. Evidence suggests that mothers and sons recognise each other: females never mate with sons (Cockburn *et al.* [2003]; Chapter 2) and sons never display to their mothers (Cockburn *et al.* [2016]; Chapter 4). It is therefore possible that the dominant males adjust their behaviour, for example in attempting to assert their dominance over unrelated helpers on the territory, allowing the breeding female greater liberty to cheat with extra-group males, or simply leading to a delay in mating between the dominant male and the female returning from an extra-group foray. Such delay could lead to biasing sperm competition - and fertilisation - in favour of the extra-group males with whom the female has mated earlier that morning during her foray.

Untangling the complex effects of unrelated helpers on mating dynamics will therefore require a detailed spatial analysis and tests of whether breeding females and/or dominant males respond differently to related *versus* unrelated helpers. For instance, given a female's dependence on assistance with parental care [Dunn and Cockburn, 1996], whether or not a female proceeds with a breeding attempt may change when faced with a reduction in the number of helpers of a particular relatedness type. There is evidence that fairy-wren females can adjust their levels of investment in eggs or nestlings in the presence of helpers [Russell *et al.*, 2007, 2008; Langmore *et al.*, 2016]. However, it is unknown whether this adjustment is affected by helper relatedness. Even though I found no evidence that helpers of different relatedness types provision differently or differentially affect nestling mass/survival, it is also possible that the reliability of assistance with offspring care (or perceived reliability) varies between helpers of different relatedness.

'Load-lightening' for the dominant male may also vary with helper relatedness. Dominant males

reduce care in the presence of helpers, but the benefits of this workload reduction are unclear [Green *et al.*, 1995; Dunn and Cockburn, 1996]. If the reduction of care is linked to increased paternity uncertainty, then I would expect dominant males to reduce care in the presence of unrelated helpers, but not in the presence of helper-sons. Dominant males show aggression towards helpers experimentally prevented from feeding [Mulder and Langmore, 1993], but again it is not known whether this aggression varies when helpers of different relatedness types are prevented from feeding young. Overall, are dominant males more aggressive towards unrelated helpers? Investigating the effects of helper relatedness on group dynamics in more detail would allow us to explore the pathway through which females increase infidelity in the presence of unrelated helpers.

A further question remains as to why dominant males tolerate unrelated helpers in their group, when female infidelity increases in their presence? Unrelated helpers are common (38% of all helpers) as 'turn-around' of breeding females is relatively high: female lifespans are shorter than those of males [Cockburn *et al.*, 2008a], and females may also divorce their social partners and move territories [Cockburn *et al.*, 2003]. Thus, dominant males are often surrounded by helpers unrelated to the breeding female. We know that dominant males punish helpers if they sing in the dawn chorus [Cockburn *et al.*, 2009, 2016] or are experimentally prevented from feeding young [Mulder and Langmore, 1993]. This suggests that the dominant males know that the presence of helpers is costly to them, and yet helpers are tolerated on the territories. Since larger groups of males do not result in more per capita visits from females [Cockburn *et al.*, 2009], there is no apparent advantage of having helpers in terms of attracting potential mates. The most plausible explanation for the tolerance is linked to the apparent asymmetry in male mating success - reproductive success is skewed towards a few select males [Cockburn *et al.*, 2016]. The high quality dominant males may tolerate helpers as they can outcompete them easily, whereas the options of the lower-quality dominant males may be more restricted. These lower-quality males may be forced to accept the presence of helpers on their territory, regardless of the costs, in order to keep the dominant position and secure some within-pair paternity. While this reproductive asymmetry may explain why dominants tolerate helpers despite the associated fitness costs, it does not explain why we see an increase in extra-group paternity in the presence of unrelated helpers.

3.4.1. Conclusions

In conclusion, my study illustrates how relatedness between individuals can potentially affect important aspects of group dynamics. I observed a substantial increase in rates of extra-group paternity in the presence of unrelated helpers, but not in the presence of helper-sons. This unexpected result undermines the constrained female hypothesis as an explanation for the widespread infidelity in this population. I also found no evidence of any difference in productivity of nests with helpers of different relatedness. I suggest that the association between infidelity and helper relatedness may be due to variation in population density, between-female

behavioural differences in dispersal/foray propensity, or due to changes in the breeding female's and the dominant male's behaviour in the presence of unrelated helpers. More generally, my results illustrate how characteristics of conspecifics, rather than just their numbers, have the potential to be important for multiple aspects of group dynamics. I note also that whilst awareness of the role of the social environment and the potential role for indirect genetic effects (IGEs) in behavioural and evolutionary ecology is growing [Bailey *et al.*, 2018], standard IGE models typically treat all interacting conspecifics as equivalent. I have shown here that particular characteristics of these conspecifics may play a crucial role in shaping reproductive patterns.

Chapter 4

Strategic male courtship and inbreeding avoidance in a cooperatively-breeding bird

ABSTRACT

While female mate choice has been studied extensively, the role that males play in mate choice has only started receiving attention more recently. Males typically invest less in reproduction and can therefore potentially sire many more offspring than females, and hence are expected to be less choosy. For example, it has been suggested that males may be more tolerant of inbreeding than females. Here, I investigate male mate choice and inbreeding avoidance in cooperatively-breeding superb fairy-wrens (*Malurus cyaneus*) though investigating strategic courtship. Analyses of pedigree data from our long-term study of the superb fairy-wrens for the years 1988-2013 showed no cases of offspring resulting from matings between mothers and sons, despite frequent social pairings between mothers and their sons, and numerous cases where mothers lived in close proximity to their sons. However, we do not know the extent to which this inbreeding avoidance is driven by female rejection of any courtship attempts by her sons, or whether males strategically choose not to pursue matings with their mothers. I collected behavioural data over two field seasons, recording which males visited and performed courtship displays to focal females. In order to complement Chapters 2 and 3, I focused on the male, rather than female, perspective, and on a different social context: mothers and sons no longer living on the same territory. I found that females were rarely visited by their sons ($<2\%$ of visits) and that sons never displayed to their mothers. Clearly, males can recognise their mothers even when living on different territories. Importantly, these results indicate that mother-son inbreeding avoidance is not driven entirely by the females. The observation that males do not attempt to court their mothers suggests that males face opportunity costs and thus exercise mate choice when deciding which females to visit and to whom they should display. This work opens up interesting avenues for further investigation into kin recognition, as well as into mutual mate choice in superb fairy-wrens, a species where mate choice was thought to be predominantly female-driven.

4.1. Introduction

An individual's choice of mate may be a fundamentally important determinant of its reproductive success and hence its overall fitness. However, understanding the factors that determine mate choice remains a challenging component of behavioural ecology [Mead and Arnold, 2004; Fuller *et al.*, 2005]. Despite clear evidence for adaptive significance of many aspects of mate choice [Andersson, 1994; Andersson and Simmons, 2006], choosing between different potential mates may also incur costs. Mate choice can be costly in terms of time and energy [Milinski and Bakker, 1992; Judge and Brooks, 2001; Wong and Jennions, 2003], or higher risks of either predation and/or being injured while distinguishing between different potential mates [Schluter and Price, 1993; Gibson and Langen, 1996; Crowley *et al.*, 1991].

Considerable attention has been given to studying female mate choice [Andersson, 1994; Mead and Arnold, 2004; Kokko *et al.*, 2003], no doubt due in part to the expectation that males will be less choosy than females, as they generally invest less in the offspring [Andersson, 1994]. However, males may also exhibit mate choice, especially when biparental care is important for the survival of the offspring [Kokko and Johnstone, 2002], but male mate choice may even be found in species without sex-role reversal [Bonduriansky, 2001; Servedio, 2007; Edward and Chapman, 2011] or in species without paternal care [Amundsen and Forsgren, 2001; Bel-Venner *et al.*, 2008; Reading and Backwell, 2007]. In monogamous species mutual mate choice is expected to arise [Andersson, 1994], but our understanding of male mate choice under polygyny is more limited.

Theoretical models indicate that in polygynous systems male mate choice is more likely to arise when female traits indicate high viability or fertility [Servedio and Lande, 2006]. However, male preference for 'arbitrary' female traits can be maintained if the choosy males are able to increase their total courtship 'amount' relative to males mating at random [Servedio and Lande, 2006; Servedio, 2007]. Moreover, empirical evidence of male mate choice is growing, showing that female characteristics such as reproductive state [Kelso and Verrell, 2002], variation in fecundity [MacLeod and Andrade, 2014], age [Muller *et al.*, 2006], body size [Sargent *et al.*, 1986; Svensson and Petersson, 1988; Wong and Svensson, 2009; Svensson *et al.*, 2010] or colouration [Amundsen *et al.*, 1997; Tigreros *et al.*, 2014] can play an important role in male mate choice.

Relatedness between individuals may also be relevant to both male as well as female mate choice decisions. Mating with relatives, i.e. inbreeding, decreases the frequency of heterozygotes (which could have higher fitness), and increases homozygosity which may lead to expression of deleterious recessive alleles [Charlesworth and Charlesworth, 1999]. The reduction of fitness of inbred offspring, or inbreeding depression [Parker, 1979], generates selection pressures that will then shape the evolution of mating patterns. Overall, inbreeding avoidance is predicted to evolve when inbreeding depression is sufficiently high, costs of mate choice are low and kin discrimination is feasible [Waser *et al.*, 1986; Kokko and Ots, 2006; Lehtonen and Kokko, 2015; Duthie and Reid, 2015, 2016]. The situation is further complicated by the fact that there are potential inclusive fitness benefits of inbreeding, which may thus favour mate choice strategies that comprise inbreeding preference [Kokko and Ots, 2006; Duthie and Reid, 2015, 2016]. Increasingly, empirical evidence indicates a range of mating 'strategies' in different populations [Szulkin *et al.*, 2013].

Theory also predicts that inbreeding strategies are sex-specific: males should tolerate higher levels of inbreeding than females [Parker, 1979; Waser *et al.*, 1986; Kokko and Ots, 2006]. This is because opportunity costs are expected to be higher for females, as the number of offspring that females can produce is lower than the number of offspring that males can sire. Thus, males can increase their inclusive fitness through inbreeding to a higher degree than females [Parker, 1979, 2006; Waser *et al.*, 1986]. However, if inbreeding depression is severe we could expect males to adjust their mate choice and avoid inbreeding [Pizzari *et al.*, 2004], especially with close

relatives. This would be particularly likely if there are also high opportunity costs for males: when choosing one female prevents the male from reproducing with another female [Waser *et al.*, 1986]. For more discussion on mating strategies and inbreeding avoidance mechanisms see Chapter 2 and the Thesis Introduction.

Kin discrimination is the ability to distinguish between related and unrelated conspecifics through direct and/or indirect familiarisation [Porter, 1988; Tang-Martinez, 2001; Nakagawa and Waas, 2004], also referred to as 'recognition by association' and 'phenotype matching' respectively. Evidence from mammalian systems suggests that inbreeding avoidance is linked to familiarity - and thus possibly perceived kinship - rather than actual kinship (e.g. spotted hyenas and rodents; see p. 139 of Clutton-Brock [2016] and references therein).

Kin discrimination may be particularly important for cooperative breeders. First, it has obvious advantages: if an individual assists kin, it can gain inclusive fitness benefits. Secondly, many social groups of cooperative breeders are composed of individuals of varied relatedness, therefore there may be more selection for mechanisms allowing the avoidance of mating with relatives. Detailed studies of kin discrimination in cooperative breeding birds are still relatively rare and many that exist focus on helping behaviour rather than mate choice. In general, birds rely on contextual information to recognise relatives [Koenig and Haydock, 2004; Nakagawa and Waas, 2004; Ihle and Forstmeier, 2013]. For example, cross-fostering experiments revealed that long-tailed tits treat all nestmates as kin, regardless of actual relatedness [Hatchwell *et al.*, 2001]. Similarly, cross-fostered Seychelles warblers and western bluebirds seem to accept their foster parents as genetic parents and help with brood-raising equally as often as genetic offspring do [Komdeur *et al.*, 2004; Dickinson *et al.*, 2016]. Therefore, the familiarity gained during early life (e.g. in the nest), as well as continued association between relatives throughout life, may be important for kin discrimination. There is some evidence that in certain species cues allowing discrimination of socially-unfamiliar kin exist; for instance, bell miners use call similarity to assess genetic relatedness to other individuals [McDonald and Wright, 2011], and Siberian jays are able to adjust their levels of aggression towards unfamiliar immigrants based on their relatedness [Griesser *et al.*, 2015]. Direct cues could be particularly useful, in terms of inbreeding avoidance, in systems with high extra-pair reproduction rates, where the familiarity does not predict relatedness perfectly.

In order to explore male mate choice in the context of relatedness, a costly male behaviour linked to mate choice should be investigated in the presence of both related and unrelated potential mates. Courtship is one such behaviour, as it is likely to be costly to males [Clutton-Brock and Langley, 1997; Cordts and Partridge, 1996; Mappes *et al.*, 1996]. In this chapter, I investigate male courtship by superb fairy-wrens (*Malurus cyaneus*) by assessing whether there is evidence for kin discrimination and inbreeding avoidance amongst males. I focus on male courtship behaviour, and in particular the potential for avoidance of inbreeding between mothers and sons in the situation where a male is not living on the same territory as his mother, so courtship would require travel to the mother's territory.

Superb fairy-wrens are cooperative breeders. Each territory has a dominant male and a breeding female who form a socially monogamous pair. In ~50% of cases the breeding pair is assisted by several subordinate male helpers. This study uses observational data of males visiting other territories and performing courtship displays in the hope of extra-pair paternity. It specifically focuses on close relatives: mothers and sons. Females may have sons on their own territory (as social partners and/or helpers) and/or sons living on other, almost always nearby, territories. The latter situation arises either when a mother has divorced and moved away to a different territory from the one on which her son is resident, or because a son has dispersed to take up a nearby breeding vacancy. When mothers and sons are on the same territory, there is no evidence of sexual interaction between them [Cockburn *et al.*, 2003], and our pedigree analyses showed no evidence of any offspring resulting from a mother-son pairing, either within-pair or extra-pair (Chapter 2 and 3)[1]. However, it is not clear to what extent this avoidance of inbreeding is driven by each sex.

In Chapter 2 the frequency of social pairings and of matings between mothers and sons was explored, while Chapter 3 investigated the effect of the presence of helper-sons on a female's extra-pair paternity mating patterns. Both these chapters therefore considered female infidelity. However, since mating strategy may vary between the sexes [Parker, 1979; Waser *et al.*, 1986; Kokko and Ots, 2006], it could be predicted that - all else being equal - males should be more tolerant of potential inbreeding than females. They may therefore be expected to visit and court their mothers. Here, mate choice and relatedness are explored from the male perspective, by testing whether sons no longer living with their mothers do or do not court their mothers.

Male superb fairy-wrens have two types of plumage: brown 'eclipse' plumage and blue-black 'nuptial' plumage (Thesis Introduction: Box 1). Most males acquire nuptial plumage in spring, at the beginning of the breeding season (September/October). Only a few males manage to moult earlier, in winter, as moult is costly [Mulder and Magrath, 1994], but these early-moulting males are preferred by females [Dunn and Cockburn, 1999; Green *et al.*, 2000; Double and Cockburn, 2003; Cockburn *et al.*, 2008a]. Male superb fairy-wrens start courtship displays once they are fully moulted into nuptial plumage [Peters *et al.*, 2001]. They may display to females for several months before the start of the breeding season [Dunn and Cockburn, 1999], but because only a few males achieve early moult, the peak frequency of displays is in September and October [Mulder, 1997]. Males devote a lot of time to displays [Mulder, 1997] in attempts to secure matings [Mulder and Magrath, 1994]. They continue displaying to females on other territories even when their own females are fertile [Green *et al.*, 1995], mainly visiting females in neighbouring territories [Mulder, 1997].

In superb fairy-wrens the rate of male displays does not appear to be associated with extra-pair success [Green *et al.*, 2000]. Instead, the date of moult into nuptial plumage is the best predictor of a male's extra-pair success [Dunn and Cockburn, 1999; Cockburn *et al.*, 2013]. However, it

[1]Since I carried out this analysis, individuals hatched after 2013 were genotyped using SNP data, providing the first evidence of nuclear family inbreeding event in this population.

is possible that in a situation when the information about the timing of the moult is unavailable to females, they rely more on the displays carried out during the breeding season [Green *et al.*, 2000]. Given the above arguments with regard to male tolerance of inbreeding, and if courtship was cost-free, males would be expected to visit and court their mothers as much as they visit and court unrelated females, due to lower opportunity costs and predicted higher tolerance of inbreeding. On the other hand, given the inbreeding depression demonstrated previously (Chapter 2), if male courtship displays involve substantial opportunity costs it is likely that they would exercise some level of strategic courtship and display selectively: in order to maximise their reproductive success, males should preferentially visit and court unrelated females.

4.2. Methods

4.2.1. Study system and fieldwork

Summary

This study is based on a wild population of superb fairy-wrens (*Malurus cyaneus*) located in and around the Australian National Botanic Gardens, Canberra, Australia (35°16 S, 149°06 E). Individual characteristics, including the timing of the moult, were recorded. During the breeding season any breeding attempts and group composition were also monitored.

The details of data collection, descriptions of what was counted as visits and displays, as well as details of how candidate pools of potential visitors were determined are given in the sections below. In short, the focus of this study was on male courtship behaviour, specifically visits and displays carried out by males, and the choice of females that a male courted. However, due to the practical difficulties of following small fast-moving birds through dense vegetation, it was not feasible to follow individual males and record whom they visited. Instead, I chose focal females who were known to have sons alive on nearby territories, and recorded all males visiting these females in specific periods. Candidate pools of potential visitors, i.e. fully-moulted males within a specified distance from the focal female's territory, were calculated for each focal female (criteria are given below). Across two field-seasons (2015 and 2016), 18 females were watched for an average of 13 watches of 30 minutes each, giving a total of 234 watches. For each watch, I recorded whether or not each of the candidate males visited, and whether or not he displayed. Note that this took into account whether a male visited, but not the number of visits - males could visit multiple times during each watch (display rate was investigated in Green *et al.* [2000]).

To illustrate sample sizes, with 18 females watched, if each female had 15 males in her candidate pool and was watched 13 times, the overall sample size would be 3510 'observations' (as $18 \times 15 \times 13 = 3510$).

Data collection

Data were collected over September-October in 2015 and 2016. Since almost all birds in the study population are colour-banded they can be identified in the field. Interactions between mothers and sons were of particular interest in this study. Therefore, all females that had sons that had finished their nuptial moult (into blue-black plumage) living close by, but not on the same territory, were targeted as focal females. This resulted in eight mothers and eleven mother-son combinations (three of the females had two sons each). In order to ensure that the analyses were not biased due to the selected birds sharing specific characteristics, ten additional females without sons present on other territories at the same time were also watched.

Data on visits and displays were collected for all females. Each female was watched between five and twenty-three times, giving an average of thirteen watches per female, and 235 watches in total. Watches were not carried out during the hottest part of the day or when it was raining, as the birds were considerably less active during these times (pers. obs.). Each watch lasted for 30 minutes, from the moment the focal female was located within her territory and the members of her social group present in the vicinity were identified. During the watch the focal female was kept in sight, and all visiting males (i.e. males from another group) and their behaviour during the visit were recorded. Incomplete watches were discarded. Previous studies indicated that several males are likely to visit to court females over a 30 minute time period.

Visits and displays

Routine censuses of birds have been used for many years to establish the territory boundaries of each female and her social group. While territory boundaries can be relaxed during the winter, territories are rigorously enforced during the period I conducted my observations. Any movement into the focal female's territory by a male from another group was counted as a 'visit'. Interactions with the focal female were recorded as a 'display' if they involved courtship behaviour, defined as one or more of the following: approaching the female, raising and fanning out of the cheek feathers, and positioning in front of the female in such a way that brown feathers are hidden and blue-black feathers are most visible to the courted female (Figure 4.1). If a visiting male did not interact directly with the female and/or was prevented from performing the courtship behaviour described above, the event was recorded only as a 'visit' and not as a 'display'. For instance, this could be the case when females were hidden in a bush or were nest building; when visiting males attempting to approach the female were chased off by the resident males; or when the visitor approached and chased the female, but did not engage in the courtship behaviour described above.

Nine visits from unbanded males were excluded from the analyses as it was not possible to ascertain where these males came from, or how old they were; eight visits from six banded visitors who resided outside of designated candidate pools (see below) were also excluded.

Figure 4.1. Main image: A superb fairy-wren male preparing to display: raised cheek and mantle feathers are visible. Insert: Side/back view of a displaying male showing black feathers being used to cover the brown wings; note the raised cheek feathers and flattened blue feathers on the top of the head. Photos: GK Hajduk

Candidate pools

For each focal female and for each watch a candidate pool of potential visitor males was constructed. The maps of the study site were digitised using *Autodesk AutoCAD for Mac* (version O.48.M.299), marking territory boundaries and calculating the geometric centres of all the territories. The distances between the centres of the territories of the focal females and the centres of neighbouring territories were measured. All males residing within 205m of the focal female's territory (measured geometric centre to geometric centre; approximately twice the radius of the biggest territory) and who had fully moulted into blue breeding plumage by the date of the watch were designated as that female's 'candidate pool', and treated as potential visitors.

One map per each month of data collection was digitised to take into account potential changes in the territory boundaries, residents, group composition *etc*. Not all males were fully moulted at the start of each season, and so the pools of candidates for each female at a given date were subject to change: males were added to the pool of candidates if and when they acquired their nuptial plumage, as males start extra-group courtship at this time. Overall, 90 unique candidate males were identified, 57 unique candidates in 2015 and 69 unique candidates in 2016, although note that each male could fall into several candidate pools (and indeed 77% of males belonged to more than one candidate pool). On average there were fifteen candidate males per female per year. The social status (dominant *vs* helper) of each candidate male could change both within

and between seasons: in 2015 there were 48 dominant candidate males and ten helper candidate males (i.e. one of the males changed his status), while in 2016 season, 54 candidates held the dominant status and twenty were helpers (i.e. there were five changes of social status).

4.2.2. Statistical analyses

I fitted generalised linear mixed models, (1) modelling `visit` as a binary response, and (2) modelling `display` as a binary response. For the `visit`, the models were fitted using all data (presented below), as well as only using the watches during which at least one visitor was observed (see Appendix C). For each candidate male I scored whether or not he visited the focal female during each watch (a total of 3379 possible visits). For the displays analysis, data for the visitors were used, i.e. whether or not a visiting male displayed to the focal female (a total of 262 possible displays). All models had the same fixed and random structures, as detailed below. Data with missing fixed effects were removed from the models and the numbers of 'observations' used are given alongside model results in the tables. Non-independence of data was taken into account in the random structure of the models.

Fixed Effects: I fitted whether the focal female was the candidate male's mother (`candidate visiting his mother?`; two-level factor: no, yes), to test whether males avoid visiting and/or displaying to their mothers; the `candidate's age` and focal `female's age` (both as two-level factors: one-year-old *vs* older) to account for any differences in experience and/or attractiveness; `distance between the candidate & female` - the distance between the centre of the candidate male's territory and the focal female's territory (continuous, rounded to the nearest metre) to account for how far each male had to travel to visit a specific female; `candidate's moult date`, as males only display once they complete the moult; `candidate's social status` (two-level factor: dominant, helper) to account for potential differences stemming from the social position within a group; the `field season year` in which the watch was carried out (two-level factor: 2015, 2016); and the `number of helpers` (continuous: 0, 1, 2) on the focal female's territory, as larger groups could potentially be more vigilant against visitors. **Random Effects:** The date of trial was fitted (`trial date`), as multiple watches were carried out each day over a total of 70 days; the focal `female's ID` was included to account for each female being observed multiple times; and the `candidate's ID` was fitted, as each male could visit multiple females and/or visit multiple times.

Additionally, as the effects of `candidate's moult date` could be confounded with the effects of the `candidate's age`, I fitted a model dropping `candidate's age` from the fixed effects (see Appendix C).

The models were fitted using the R package *MCMCglmm* [Hadfield, 2010], with binomial error distributions. I aimed for effective sample sizes of 2000, ensuring that they were always above 1000. Terms were considered statistically significant when the 95%

credible intervals (CIs) did not span zero and when *pMCMC* values < 0.05 (*pMCMC* = the number of simulations in which the parameter estimate was greater or less than zero, corrected for number of *MCMC* samples). Parameter expanded priors were used (`V = 1, fix = 1, nu = 1, alpha.mu = 0, alpha.V = 1 × 1000`), and the analyses were run for 1.3×10^6 iterations with a burn-in of 3×10^5 and a thinning interval of 500.

4.3. Results

Visits. Out of the total of 90 males identified as potential candidate mates for the focal females, 51 (57%) were observed as visitors in a total of 262 visits (out of a total of 3379 possible visits). The number of visits I observed per female in a season (either 2015 or 2016) ranged from 0 to 32 (*median* = 8), and, on average, I observed visits to a female by 30% of her total pool of candidate males in a given season (*min* = 0%, *max* = 71%). The number of females visited by each visitor in a given season ranged from 1 to 5 (*median* = 1), and 41% of unique visitors were observed visiting more than one female.

Of the total of 262 visits observed, there were only five instances of a son visiting his mother's territory (three different sons visiting their mothers on one/two/two occasions), although candidate males who were sons were observed visiting other females (a total of 58 times). The GLMM showed that candidate males were less likely to visit their mothers than to visit unrelated females (Table 4.1, Table C.2).

Older candidate males were more likely to visit females than were one-year-old candidate males. The mean distance between the centre of the female's territory and the centre of the territory of the males who visited her was 105m, and males living further away were less likely to visit than males close by (Table 4.1). There was also an indication that males who had moulted earlier in the year were more likely to be observed as visitors than were those who moulted later ($p = 0.054$), and that visits were more likely in 2016 than in 2015 ($p = 0.053$). As the effects of the moult date could be confounded with age I fitted an additional model dropping candidate's age - the effect of the moult date was strengthened considerably ($p = 0.005$, Table C.1), and so was the effect of season ($p = 0.003$, Table C.1). The age of the focal female and the number of helpers on her territory were not associated with visits. Even though only 11% of visits were carried out by helpers, as opposed to dominant males, the social status of the candidate male was not associated with visits (Table 4.1, Table C.2). Variance components in the model indicated substantial differences between females in their probability of being visited, and between candidate males in their probability of being observed as visitors.

Displays. Over half of the visits resulted in displays (144/262 i.e. ~55%), with the percentage varying from 0 to 100% across females per season (*mean* = 52%; *median* = 56%). The number of displays ranged between 0 and 21 displays per female/season (*median* = 4). Overall, the number of males from whom a female received displays varied from 0 to 9 (*median* = 3), and on average

Table 4.1. What affects the probability of a female being visited by a candidate male? The model was fitted in *MCMCglmm* using data from all trials, including watches during which no visitors were observed (235 watches of 18 focal females, with a total of 3379 possible visits). 'Number of helpers' is the number of helpers residing on the focal female's territory at the time of the potential visit.

	visited (no, yes)	
Fixed effects	**Estimate (95% CI)**	**P**
intercept	159.68 (-10.18, 311.79)	0.057
number of helpers	-0.26 (-0.66, 0.15)	0.230
field season year (2015, 2016)		
2016	3.49 (-0.18, 6.68)	0.053
candidate's social status (dominant, helper)		
helper	-0.17 (-0.86, 0.56)	0.648
focal female's age (1yo, older)		
older	-0.49 (-1.39, 0.32)	0.258
candidate's moult date	-0.01 (-0.02, 0.001)	0.054
distance between the candidate & female	-0.02 (-0.03, -0.02)	**<0.001**
candidate's age (1yo, older)		
older	0.95 (0.04, 1.79)	**0.026**
candidate visiting his mother? (no, yes)		
yes	-1.85 (-2.99, -0.73)	**0.001**
Random effects	**Variance (95% CI)**	
trial date	0.22 (<0.001, 0.47)	
female ID	0.48 (0.09, 1.09)	
candidate ID	0.71 (0.20, 1.28)	
residual	n/a	
Sample size	3379	

64% of visitors (unique visitors per female/season) displayed to a female at least once during their visits. (Note: since males may belong to several candidate pools, they may be counted as a 'unique visitor' for more than one female influencing the 64% average quoted; overall - at the population level - 75% of all males that visited females displayed at least once.) Older visitors were more likely to display than were younger visitors, but none of the other fixed effects were associated with displays (Table 4.2). Visiting males never displayed to their mothers, though 60% of visitors who were sons of one of the target females were observed in displays to other females (on 33 occasions).

Table 4.2. What affects the probability of a visitor displaying to a female? The model was fitted in *MCMCglmm* using data for the observed visiting males (32 males visiting 17 females in 128 watches, $n = 262$ visits).

	displayed (no, yes)	
Fixed effects	**Estimate (95% CI)**	**P**
intercept	81.18 (-180.79, 353.12)	0.561
number of helpers	-0.08 (-0.94, 0.82)	0.900
field season year (2015, 2016)		
2016	0.98 (-4.98, 6.64)	0.749
candidate's social status (dominant, helper)		
helper	0.89 (-0.59, 2.38)	0.229
focal female's age (1yo, older)		
older	-0.17 (-1.78, 1.39)	0.830
candidate's moult date	-0.01 (-0.02, 0.01)	0.554
distance between the candidate & female	0.01 (-0.003, 0.02)	0.156
candidate's age (1yo, older)		
older	2.05 (-0.08, 4.02)	**0.044**
candidate visiting his mother? (no, yes)		
yes	-12.81 (-22.99, -2.38)	**0.001**
Random effects	**Variance (95% CI)**	
trial date	0.66 (<0.001, 1.84)	
female ID	1.10 (<0.001, 3.03)	
candidate ID	0.35 (<0.001, 1.25)	
residual	n/a	
Sample size	262	

4.4. Discussion

This study investigated male mate choice in superb fairy-wrens by testing for evidence for close-kin discrimination and inbreeding avoidance. I focused on males visiting territories of females of different relatedness, specifically males visiting their mothers, and on whether they then carried out courtship displays during their visits.

Male strategic investment in courtship in polygynous species is still understudied. In superb fairy-wrens, copulations are controlled by females: in particular, extra-pair copulations occur when females carry out early morning forays to the territories of their preferred extra-pair males and then solicit copulations [Double and Cockburn, 2000]. The role of male strategic courtship display in superb fairy-wrens is less well understood. If there is little opportunity cost for a male in mating with a given female, males should attempt to secure as many matings as possible, regardless of any characteristics of the female. However, if pursuit of mating with one female comes at the cost of mating with another, targeting of particular females will be beneficial. My previous work (Chapter 2) found evidence for the presence of inbreeding depression in the study population, indicating that there are costs associated with inbreeding in this species. If this inbreeding depression is sufficient to outweigh inclusive fitness benefits of mating with relatives, and if there are opportunity costs involved in male courtship, males should avoid mating with relatives. The analyses in Chapter 2 indicated that females socially paired to their sons use extra-pair matings to avoid inbreeding with immediately-familiar close kin. The aim in this study was to explore this inbreeding avoidance further, and test whether it is driven by male strategic decisions as well as female choice.

Although I only observed a small proportion of all possible combinations of candidate males visiting focal females, more than half (57%) of the potential candidate males were recorded as visitors to at least one female, and up to 71% of the males in a focal female's candidate pools were recorded visiting her. Despite this evidence of substantial visiting activity, males were significantly less likely to visit the territories of their mothers: there were only five instances of such visits (5/262 i.e. $<2\%$ of visits), and a male was significantly less likely to be observed visiting a given female if she was his mother. Further, importantly, sons were never observed displaying to their mothers, but they did display to other females. Therefore, in superb fairy-wrens, the avoidance of close inbreeding is not driven entirely by the females alone: males seem to be avoiding displaying to their mothers, suggesting that they are avoiding inbreeding too, despite the theoretical models predicting that males ought to be more tolerant of inbreeding than females. These results imply that any inclusive fitness benefits of inbreeding are not sufficiently high to outweigh the costs of inbreeding depression for males. Additionally, these results indicate that courtship is costly for the superb fairy-wren males and suggest that there may be opportunity costs of male courtship too. For instance, it is possible that visiting and displaying to mothers would reduce the amount of time available to court unrelated females. If true, such costs would have to be considered alongside the costs of inbreeding depression - and

thus make inbreeding avoidance more beneficial for the males.

In Chapters 2 and 3, I demonstrated that when males live on the same territory as their mothers (either as social partners or as helpers), they do not breed together. I wanted to test whether there is evidence for such inbreeding avoidance when mothers and sons live on different territories, as either sex may move territories (e.g. a mother may divorce and move away from a territory, and sons may disperse to take up nearby empty vacancies). Does kin discrimination and inbreeding avoidance continue when mothers and sons no longer share a territory? This study provides further evidence for the existence of behavioural (pre-copulatory) inbreeding avoidance between closely-related individuals across a new context: birds living on separate territories, and confirms the continuation of kin discrimination and inbreeding avoidance under these circumstances. Therefore, there is now evidence for both within- and between-group inbreeding avoidance, as well as evidence that both sexes exercise mate choice with regard to relatedness.

4.4.1. Male *vs* female choice

How important is male choice of whom to court, especially compared to female preference? In superb fairy-wrens, males visit and court females throughout the day, but matings do not occur during or immediately after displays. Instead, females control extra-pair reproduction: they carry out pre-dawn forays into the territories of their preferred extra-pair mates and solicit copulations there [Double and Cockburn, 2000]. It has been shown that a male's date of moult into breeding plumage is the most accurate predictor of the extra-pair reproductive success of males [Dunn and Cockburn, 1999; Cockburn *et al.*, 2013], suggesting that females may use that to judge male quality and inform their choice of mate. It is possible that the period of days over which a male displays provides the female with additional information; for instance, it not only reminds her of the male's existence, but reinforces that he is still alive and available for mating. Additionally, it is still unclear how females determine where their preferred males reside: one hypothesis is that some aspect(s) of the display conveys the directional information necessary for the female to locate the male's territory (Cockburn *et al.* [2013]; A. Cockburn pers. comm.). Therefore, even though males may not gain access to the females during the courtship displays, it is possible that males exercise mate choice through these displays, by choosing which females are provided with the information about their state and location.

4.4.2. Why visit?

Overall, over half of all visits (~55%) resulted in displays, supporting the notion that the purpose of at least a large proportion of the visits is linked to courtship. While some visitors showed no signs of attempting to display, 75% of all visitors displayed at least once. It is also possible that some of the males visited in order to display to the focal female, but were unable to do so due to the female not being 'accessible'. For instance, many of the visitors were chased away by the resident males on the focal territory soon after approaching the female or even during

Figure 4.2. A superb fairy-wren male carrying a yellow wattle petal. Photo courtesy of Gerard Jenkins, reproduced with permission.

the approach. There were also seven instances in which the visitor was carrying a yellow petal (amounting to 29% of the observed petal-carrying events) (Figure 4.2), but did not display due to the female being hidden in a bush and nest-building. These were recorded as visits, but not as displays, since the males did not interact with the focal female. However, since petals are used in displays [Mulder, 1997], it seems reasonable to infer that these petal-carrying males intended to perform a display. Therefore, the proportion of visits for which the purpose was to engage in a courtship display could have been underestimated. However, males may potentially visit females for reasons unrelated to courtship, and there may be other factors affecting the likelihood of a visiting male carrying out a courtship display.

For example, while displays were equally likely in both seasons, the data indicated that visits were more likely in 2016 than they were in 2015. It is not immediately obvious why that would be the case. However, the weather conditions during the two field seasons were quite different. The 2016 field season was much wetter (2015: 17mm of rainfall in September, 18mm in October; 2016: 154mm in September, 48mm in October). Furthermore, females started their nesting attempts earlier in 2016 than in 2015: in 2016 females were observed nest building a full two weeks earlier; egg incubation began twelve days earlier; and the first nestling hatched sixteen days earlier than in 2015. It is possible that males were visiting females in order to examine at which stage in the breeding cycle the females were (nest-building? egg-laying? incubating?), which could potentially allow them to estimate female fertility.

It is also possible that males attempt to assess the quality and/or fecundity of the females in their neighbourhood by visiting and comparing several females: 41% of visitors were observed visiting more than one female. Moreover, visitors could be monitoring close-by territories for vacancies, waiting for an opportunity to take over a dominant position (if the male is a helper) or to move over to a higher-quality territory/group.

⋆⋆⋆

A male's decisions regarding whether to visit and/or display to a particular female are likely to be affected by a range of other factors (besides the relatedness of individuals), such as the social and biophysical environment, characteristics of the females, and characteristics of the male himself. I discuss the role of these different factors in shaping male courtship behaviour below.

4.4.3. Environmental factors

Visits are likely to be costly to a male in terms of time used (that could be spent otherwise, e.g. foraging), energy expended and increased risk of injury/mortality. We would therefore expect to see a negative association between the distance and probability of visiting. Indeed, candidate males that had to travel further in order to visit a focal female were less likely to be recorded as visitors. However, once a male was observed visiting a given female, the distance he had travelled was not associated with the probability of him displaying during the visit.

Habitat characteristics, such as the type, structure and the density of ground cover, were not explicitly considered in this study, but could be of interest when exploring male mate choice and in establishing what determines which females are visited and displayed to. For instance, males may be less likely to travel through large open spaces with nowhere to take shelter from a predator or to rest (fairy-wrens are poor fliers, pers. obs.), than they are to travel through areas of shrubbery that offer cover. Additionally, dense vegetation could make it difficult for males to locate the female within her territory, particularly on bigger territories. Since I followed the focal females and recorded males that came close to them, it is possible that there were males that entered areas of the focal territories away from the female's location. Such males would not be observed and would not be recorded as visitors despite venturing into the female's territory.

Evaluating the role of the environmental characteristics would require not only a detailed assessment of the habitat on the study site, but also knowledge of the route taken by the travelling male. In order to do that males would have to be closely followed or, ideally, equipped with transmitters so that their movements could be recorded and mapped out. However, previous attempts at tagging males with 0.4g transmitters revealed that the males cease to display when carrying the extra weight (A. Cockburn pers. comm.); therefore using such transmitters to explore courtship displays was not feasible, though females always made mating forays when

bearing a transmitter [Double and Cockburn, 2000].

I found no evidence that the social environment, in terms of the numbers of helpers present on the focal female's territory, was associated with the probability of visits or displays. This was somewhat surprising as all resident males on a territory (both dominant and helpers) defend the territory and chase away intruders, so it might be expected that larger groups received fewer visits and displays. However, although superb fairy-wren females may have up to four (or very rarely five) male helpers in a group, the females observed in this study had relatively few helpers: in 2015, out of the nine females monitored eight had no helpers and one had one helper, while in 2016, out of the 15 females monitored, eight females had no helpers, five females had one helper and two females had two helpers. Ideally, it would therefore be worth exploring whether higher numbers of helpers have an impact on visits/displays, in case there is a threshold at play and larger groups are different from smaller groups/pairs. Given my results in Chapter 3, indicating dramatic differences between the effect of related *vs* unrelated helpers on rates of extra-pair paternity, I also tested whether either visitation or display rates varied with the presence of helpers of different types, but found no effect (results not shown).

4.4.4. Male characteristics

Male reproductive effort may be expected to increase with age [Hayes *et al.*, 2013], and older males may also have more experience and/or knowledge, e.g. they may have more information about where the females are located. In this study, older males were more likely to visit, and the models also suggested that older visitors were more likely to display, than were one-year-old males.

The date of a male's moult into the nuptial blue-black plumage is known to be a good predictor of extra-pair reproductive success in this population of superb fairy-wrens [Dunn and Cockburn, 1999; Cockburn *et al.*, 2013]. If early-moulting males are of higher quality, they could be expected to visit and/or display more often than lower-quality males that moult later in the year. The analysis suggested an association between a male's time of moult and his visitation rates, possibly indicating that males moulting earlier in the year were more likely to be observed as visitors than were later-moulting males. However, the moult date of the visitors was not associated with their probability of carrying out courtship displays. There was also no effect of a candidate male's dominance status (whether he was a dominant male or a helper on his own territory) on his probability of visiting and displaying.

4.4.5. Female characteristics

My models indicated variation between females in how likely they were to be visited. There was also considerable variation in whether females were displayed to by their visitors: I observed no displays for some females despite recording visits, while for other females visits always ended in displays (*mean* = 52%; *median* = 56% of visits resulting in displays). Males may be attracted

to more experienced or fecund females (e.g. Jones *et al.* [2001]; Amundsen *et al.* [1997] and references therein). Therefore, we could expect older females to be visited/displayed to more than one-year-old females, but I found no support for an association between female's age and visits or displays.

4.4.6. Further work and conclusions

The results presented here suggest several aspects of mate choice and kin discrimination in the superb fairy-wrens that would be worth investigating. I suggest two possibilities below. For instance, one could attempt to disentangle whether males recognise their mothers in all contexts, or if they remember their natal territories and avoid displaying to females resident on these territories. In this species, females may divorce their social partners and move territories [Cockburn *et al.*, 2003]. Therefore, data on female movements over the years would be required to explore this line of enquiry. It could then be assessed whether males visit and display to unrelated females when such females live on the male's natal territory, or if males avoid all females on their natal territory regardless of relatedness. Equally, it could be tested whether males refrain from displaying to their mothers once mothers have moved to a new territory. In addition to the above, the role of familiarity could be explored in more detail. To do that, other close relatives, such as brothers and sisters, could be considered. First, the behaviour of brothers in the presence of sisters could be examined in the context of visits and courtship. Secondly, spatiotemporal factors could be evaluated. Superb fairy-wrens are multi-brooded and long-lived: they can raise several broods of offspring within each breeding season and breed for several years [Cockburn *et al.*, 2008c]. This means that siblings from one nest, different nests within one season, or different seasons could be compared, if enough data were available. Siblings from these various combinations would have different levels of familiarity with each other - they could have been raised together or might have not met on the natal territory. While the behaviour of siblings has not been investigated in detail, there were no offspring resulting from sibling pairings in the pedigree used here. Both the suggested above studies would require substantial long-term work, with observations spanning several years, in order to acquire a sufficient amount of data. It was not feasible to carry these out over the duration of this PhD. However, the role and mechanisms of sibling recognition in birds have been suggested as an area needing more work [Nakagawa and Waas, 2004], and the outlined analyses could inform our understanding of the role of kin discrimination/sibling recognition in mate choice, including inbreeding avoidance.

In conclusion, even though theory predicts that males should tolerate higher levels of inbreeding than females do, my study of male courtship behaviour in superb fairy-wrens indicates that sons rarely visited their mothers and never displayed to them, indicating avoidance of close inbreeding. While there may be several reasons for males to visit the territories of females, and multiple factors affecting visits and/or displays, the data presented here support the notion that visits are linked to male mate choice through strategic courtship. Furthermore, the results of this study suggest that there are opportunity costs to male visits and displays, and confirm

that sons living apart from their mothers are still able to recognise them as close relatives. By investigating mate choice and mating strategy from the perspective of males, this chapter complements Chapters 2 and 3 of this thesis. Additionally, this work provides a base for further enquiry into mating strategies, kin discrimination and mate choice in the superb fairy-wrens - understanding of which is integral to understanding the reproductive success and fitness of individuals.

Chapter 5

Selection on body size: genetic and non-genetic determinants of associations between size and survival

ABSTRACT

Environmental and biological factors determine the phenotypic variation between individuals, understanding of which is crucial to our understanding of selection and microevolution. Body size is a commonly investigated morphological trait in these types of studies. In the superb fairy-wrens (*Malurus cyaneus*), mass and survival are correlated at the phenotypic level, giving an appearance of positive selection on body size. Since mass is also heritable, according to the breeder's equation, a response to selection should be expected under these circumstances - yet it has not been observed. The Robertson-Price identity provides an alternative means of predicting the response to selection, as the additive genetic covariance between the trait and fitness. Here, I use bivariate mixed effects animal models to partition the (co)variance between the two traits of interest, nestling mass and survival from fledging to independence, and to investigate its genetic and non-genetic components. I found no evidence of any genetic covariance between the traits. However, I demonstrated that temporal factors likely play a crucial role in this population: the correlations between mass and survival were strongest due to the effects of differences between hatch date intervals, and to differences between cohorts. I suggest that these differences are likely due to variation in weather conditions, resource availability and predation pressure experienced by the superb fairy-wrens. The results imply that nestling mass is not having a causal effect on survival, and hence that we should not expect the observed phenotypic association to drive an evolutionary response between the two.

5.1. Introduction

Understanding of natural selection and adaptation are core in the field of evolutionary biology. There is good evidence for contemporary microevolution [Hendry and Kinnison, 1999; Bone and Farres, 2001; Kinnison and Hendry, 2001; Reznick and Ghalambor, 2001]. The breeder's equation is commonly used to predict the evolutionary response of a heritable trait under selection [Lush, 1937; Lynch and Walsh, 1998; Roff, 2002]. The equation is:

$$R = h^2 S \tag{5.1}$$

where R is the evolutionary response, h^2 is heritability of the trait of interest and S stands for the selection differential, representing directional selection [Lush, 1937; Falconer and Mackay, 1996]. However, studies that report non-zero values for both of these elements - and expect change over time - do not always find a response to selection, particularly in natural populations [Merila *et al.*, 2001c; Kruuk *et al.*, 2003; Wilson *et al.*, 2007].

Body size is commonly used as a morphological trait of choice in studies investigating selection

and evolutionary change, likely due to the relative ease with which it can be measured. For instance, body size/mass was predicted to evolve in the snow goose (*Anser caerulescens*; Cooch *et al.* [1991]; Cooke *et al.* [1990]), barnacle goose (*Branta leucopsis*; Larsson *et al.* [1998]), red deer (*Cervus elaphus*; Kruuk *et al.* [2000, 2002b]), collared flycatcher (*Ficedula albicollis*; Merila *et al.* [2001a,b]; Alatalo *et al.* [1990]; Kruuk *et al.* [2001]), and sheep (*Ovis aries*; Milner *et al.* [1999, 2000]). However, even though these studies investigated heritable traits that appeared to be under directional selection (*i.e.* both elements of the breeder's equation were satisfied), the expected response to selection was not found [Merila *et al.*, 2001c].

Possible explanations for this phenomenon are reviewed by Merila *et al.* [2001c], and include: (i) biased estimates of heritability; (ii) spatial and/or temporal fluctuations of selection; (iii) selection acting on non-heritable phenotype components; (iv) constrained evolution due to selection on correlated traits; (v) changing environment masking the genetic response to selection; and (vi) lack of statistical power. Morrissey *et al.* [2010] discuss how the assumption of causation between trait and fitness is often violated, especially in wild systems. Use of mixed effects animal models reduces the likelihood of bias by allowing more accurate estimation of variances, breeding values, and genetic correlations, as well as more efficient use of existing data.

The Robertson-Price identity provides an alternative means of predicting the response to selection [Price, 1970; Robertson, 1996]. It demonstrates that additive genetic covariance between the trait and fitness has to be taken into account in order to produce sensible predictions about the response to selection [Merila *et al.*, 2001c]. A multivariate approach allows direct estimation of the genetic covariance between trait and fitness [Morrissey *et al.*, 2010], by including fitness as one of the model's response traits.

It is important to note that even when all of the relevant quantitative genetic parameters are estimated accurately, a lack of response to selection may be plausible. When an association between two traits, X and Y, is observed, there are three possible scenarios:

(i) X has a causal effect on Y

(ii) Y has a causal effect on X

(iii) a third variable could cause both X and Y to change

Therefore, even when a relationship between a trait and fitness is observed, it may not necessarily be due to a causal effect of the trait on fitness. It is also possible that unmeasured and/or non-heritable factors could be creating the appearance of correlation between the trait of interest and fitness, which is equivalent to the trait not having a causal effect on fitness.

To summarise, while there may be an overall association between a trait, such as size, and fitness, suggesting that size is under selection, this apparent selection does not always generate an evolutionary response. Therefore, predictions based on the breeder's equation will not be

accurate if the association is not present at the genetic level, with size having a causal effect on selection. As a corollary, in order to investigate whether genes affecting size affect fitness, it is necessary to partition the overall phenotypic covariance between size and fitness into its genetic and non-genetic components.

Long-term studies of natural populations are well suited to this type of enquiry, as information about the relatedness between individuals, their survival, as well as measurements of morphological traits, are often collected over a number of generations. The superb fairy-wren population (*Malurus cyaneus*) studied in this thesis fits all these criteria: it has a genetically-informed pedigree, regular census provides information on individual survival, and a morphological trait - nestling mass - was measured [Cockburn *et al.*, 2003]. Furthermore, I have shown that mass is heritable and appears to be under directional selection: heavier individuals have a higher probability of survival (Chapter 2). According to the breeder's equation, body size should therefore be predicted to show an evolutionary response. Yet there is no evidence that the mean mass has increased over time in this population [Kruuk *et al.*, 2015].

Superb fairy-wrens are cooperative breeders, with exceptionally high levels of extra-pair paternity [Cockburn *et al.*, 2016]. Breeding pairs may be assisted by up to four (exceptionally five) male helpers, which provision the offspring [Cockburn *et al.*, 2008b]. In this chapter, I use 26 years of data and bivariate mixed effects animal models, which allow me to utilise information from the multigenerational pedigree available for this population (up to 15 generations in depth). I explore how much of the phenotypic association between mass and survival is driven by genetic, non-genetic, temporal and spatial effects, while taking into account the social environment of the superb fairy-wrens. This allows me to test whether there is selection on mass at the genetic level or the association with fitness is predominantly at the non-genetic level and therefore not expected to result in an evolutionary response.

5.2. Methods

5.2.1. Study system

This chapter uses long-term data (1988-2013) from a population of superb fairy-wrens (*Malurus cyaneus*) located in and around the Australian National Botanic Gardens, Canberra, Australia (35°16 S, 149°06 E) [Cockburn *et al.*, 2003]. Birds in the study population were monitored all 52 weeks of the year [Cockburn *et al.*, 2003]. There were usually 3-4 nestlings in a brood [Cockburn *et al.*, 2008c]. Between 5 and 8 days after hatching the nestlings were banded, weighed and had a blood sample taken for genotyping purposes. Individuals were followed throughout their lives when possible (some birds disperse out of the study area and thus cannot be monitored). Further details of this study system are given in previous chapters, with paternity assignment and pedigree reconstruction described in Chapter 2/Appendix A.

5.2.2. Statistical analyses

The aim of this analysis was to estimate the additive genetic covariance and correlation between two traits: mass and survival. This analysis also allows for partitioning of phenotypic variance, as the phenotypic variance can be decomposed into additive genetic variance and residual (environmental) variance. Both traits, nestling mass and survival from fledging to independence, were examined separately in Chapter 2 using univariate animal models. These univariate models informed the fixed and random structure of bivariate models presented here.

I fitted individual-level generalised bivariate models using a Bayesian framework implemented in the *R* package *MCMCglmm* [Hadfield, 2010] to allow for binomial error distribution of survival. Bivariate models allow estimation of among-individual variance for each of the two traits of interest, as well as estimation of covariance and correlations between the response traits. Fixed effects can be fitted for both response traits together or separately as needed.

I used `nestling mass` (Gaussian errors) and `survival` from fledging to independence (binomial errors) as the response variables. I ran this analysis in three stages: **(1)** estimating overall phenotypic (co)variances between the two traits with the first model, with minimal (essential) fixed effects; while models in steps **(2)** and **(3)** were run as mixed animal models - using the pedigree information to partition (co)variances, first without and then with additional fixed effects, accounting for characteristics which I expected might contribute to the random effects. This approach allows me to calculate the overall (co)variance in a phenotype (Model 1), and then decompose it into its particular sources in Models 2 and 3 - identifying the sources of (co)variance, allows identification of what makes individuals different from each other. Additionally, the latter two models can be compared to ensure that contributions of the variance components were not overestimated by exploring the amount of variance explained by the fixed effects *vs* the random effects. Details of the models are given below.

For all these models the effective sample sizes for specific parameters varied due to autocorrelation, but I ensured that they were always above 1000. I considered terms to be statistically significant based on 95% CIs (credible intervals) not spanning 0 and *pMCMC* values (the number of simulations greater or smaller than 0 corrected for number of *MCMC* samples) calculated by *MCMCglmm* being < 0.05. In each of the models, data on 3808 individuals hatched in 1472 broods were used. All random effects were fitted for both response traits using 'unstructured' (`us`) covariance matrices. Residual variation was set using `rcov` with an unstructured covariance matrix. Since survival was a binary trait, its residual variance was fixed to 1.

Model 1. Baseline model: baseline fixed effects and no random effects

This model estimated the overall phenotypic variance within each trait of interest and the overall phenotypic covariance between them. Only baseline (essential) fixed effects were fitted to

account for differences between the sexes, and for variation stemming from protocols used to collect mass data (`nestling age` and ' `1992` '). No random effects were included at this stage.

Baseline Fixed Effects: For each response trait, `sex` of nestling (male, female) was fitted to account for differences in size between males and females. For mass, `nestling age` at measurement (continuous, in days; as a quadratic function) was fitted because pragmatic considerations meant that nestlings were weighed at different ages (days 5-8) and hence at different stages of their development; and additionally, I fitted a two-level factor ' `1992` ' (pre-1992, 1992+) to account for the introduction of a new weighing protocol in 1992, which changed the time of day at which nestlings were weighed [Kruuk *et al.*, 2015].

Model details: The model was run for 1.3×10^6 iterations, with a burn-in of 3×10^5 and thinning interval of 500. Parameter expanded priors were used (`V = 2, nu = 1.002, fix = 2`).

Model 2. Baseline fixed effects and all random effects

The observed phenotypic variation may come from differing sources. In order to explore components of variance in each trait, random effects were added to the model. This allowed partitioning of the overall phenotypic (co)variance estimated in Model 1 into its components.

Fixed Effects: `sex` of nestling (male, female; fitted for both traits), `nestling age` at measurement (continuous, in days; as a quadratic function; fitted for mass), and the two-level factor ' `1992` ' of protocol change year (pre-1992, 1992+; fitted for mass) were fitted as described above.

Random Effects: I fitted `nest ID` to account for any similarities across multiple offspring from the same brood; an `additive genetic effect` (with covariance structure determined by the pedigree) to test for covariance between relatives [Kruuk, 2004]; and a multi-level factor of `cohort` to represent inter-annual variation (1988-2013: the '2013' cohort incorporates nestlings from August 2013 through to March 2014 *etc*.). Finally, I represented intra-annual temporal variation across the breeding season by fitting a multi-level factor of `hatch date` interval (split into twelve two-week intervals, between 23 September and 15 March).

Model details: The model was run for 5.2×10^6 iterations, with a burn-in of 1.2×10^6 and thinning interval of 2000. Parameter expanded priors were used: `V = 2` , `nu = 1.002` `fix = 2` ; `V = 2` , `nu = 2` , `alpha.mu = rep(0,2)` , `alpha.V = 2 × 1000` .

Model 3. Full model: all fixed effects and all random effects

In the final model, I included all fixed effects of interest in addition to the baseline fixed effects and the random effects fitted in Model 2. This ensured that contributions of the variance components were not overestimated and allowed me to explore the amount of variance explained by the fixed effects *vs* the random effects.

Fixed Effects: `nestling age` at measurement (continuous, in days; as a quadratic function), and the two-level '`1992`' factor of protocol change year (pre-1992, 1992+) were fitted for `mass` as described above. The `sex` of nestling (male, female) was fitted for both traits. For each trait, I also fitted: the `inbreeding coefficient` of each individual, to account for inbreeding depression; the number of `helpers` (as a three-level factor: 0, 1 and 2+; where 2+ level consisted mainly of 2 helpers with some pairs assisted by 3 or 4 helpers); `brood size` (the number of nestlings, 3-5), to account for the variation in the amount of care provided to the individual nestlings. Finally, `within-pair status` (a two-level factor: WP *vs* EP) was fitted for both traits.

Random Effects: `additive genetic effect`, `nest ID`, `cohort` and `hatch date` interval were fitted as described above.

Model details: The model was run for 5.2×10^6 iterations, with a burn-in of 1.2×10^6 and thinning interval of 2000. Parameter expanded priors were used (`V = 2`, `nu = 1.002`, `fix = 2; V = 2, nu = 2, alpha.mu = rep(0,2)`, `alpha.V = 2 × 1000`).

⋆⋆⋆

Proportional contributions of the individual variance components (R^2) were estimated as the ratio of each variance component to the total phenotypic variance (the sum of all the variance components). Thus the proportion of variance in mass explained by variance component *i* was:

$$R^2_{mass(i)} = \frac{V_{mass(i)}}{V_{mass(total)}} \tag{5.2}$$

Since the residual variance for survival was fixed to 1, the percentages of the explained survival variance accounted for by each component reported were calculated using the sum of all the non-residual variance components as the denominator. Therefore, the proportion of variance in survival explained by variance component *i* was:

$$R^2_{survival(i)} = \frac{V_{survival(i)}}{V_{survival(total\ of\ non-residual\ components)}} \tag{5.3}$$

These effects are presented as percentages in the text of the Results (Section 5.3).

I used the `us` structure to estimate (co)variances at the among-individual level (random effects) and the within-individual level (residual level; `rcov`). Variances (V) and covariances (Cov) were returned by the models. Correlations ($Corr$) between mass and survival for each of the variance components were calculated separately using variances and covariances estimated by the models. This was done as follows, for the correlation among the variance component *i*:

$$Corr_{(i)} = \frac{Cov_{mass,\ survival(i)}}{\sqrt{V_{mass(i)} * V_{survival(i)}}} \tag{5.4}$$

5.3. Results

Overall, 60% of females and 61% of males survived from fledging to independence, and there was an association between mass and survival at the phenotypic level (Figure 5.1).

To recap the relevant results of Chapter 2: the univariate models provided evidence for inbreeding depression in nestling mass (6.5%; Table A.9), but not in fledgling survival, despite heavier individuals having higher survival. The heritability of nestling mass was estimated as 18.9% (±3.2% SE).

Results for the three bivariate models exploring the relationship between nestling mass and fledgling survival probability are presented in three separate tables below. The tables include variance component *estimates* (on the diagonal), *covariances* (below the diagonal) and *correlations* between the traits (above the diagonal). These are given for the genetic and non-genetic covariances between mass and survival: additive genetic effect, as well as nest ID, hatch date, cohort, and residual effects (note: the model in Table 5.1 had only residual variance fitted).

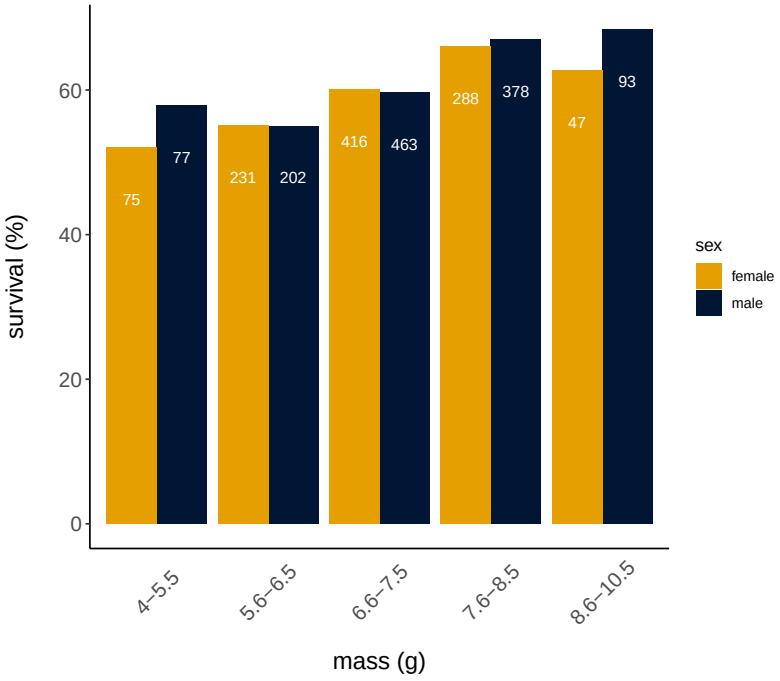

Figure 5.1. There was a positive association between mass and survival from fledging to independence for both sexes. Total numbers of individuals in each group are given within the bars.

5.3.1. Model 1. Baseline model: baseline fixed effects and no random effects

Fixed Effects: Nestling mass increased with the age at which the measurements were taken. Males were heavier than females, but there were no differences in survival between the sexes (Table 5.1).

The residual variance for mass was 0.51 (95% CIs: 0.49, 0.53) and the residual variance of survival was fixed to 1 (since it was a binary trait). The overall phenotypic covariance between nestling mass and fledgling survival to independence was 0.06 (95% CIs: 0.04, 0.09), and the correlation between the two traits was 0.09 (95% CIs: 0.05, 0.13).

Table 5.1. Baseline model of nestling mass and survival from fledging to independence (Model 1). The fixed effects are: the sex of the nestling, nestling age at measurement, and change in weighing protocol in 1992. The model estimates are based on posterior means, 95% credible intervals (CIs) are given in brackets, and *p*-values are based on *pMCMC*.

	nestling mass		survival from fledging to independence	
Fixed effects	**Estimate (95% CI)**	**P**	**Estimate (95% CI)**	**P**
intercept	-3.35 (-5.28 -1.43)	<**0.001**	0.25 (0.19, 0.31)	<**0.001**
1992 (1992+, pre-1992)				
pre-1992	0.48 (0.36, 0.60)	<**0.001**		
nestling age	2.00 (1.46, 2.56)	<**0.001**		
nestling age^2	-0.08 (-0.11, -0.04)	<**0.001**		
sex (female, male)				
male	0.16 (0.11, 0.20)	<**0.001**	0.04 (-0.04, 0.12)	0.344
Random effects	**Variance-Covariance-Correlation Matrices (95% CI)**			
	mass		survival	
residual variance				
mass	0.51 (0.49, 0.53)		0.09 (0.05, 0.13)	
survival	0.06 (0.04, 0.09)		fixed to 1.00	
Sample size	3808			

5.3.2. Model 2. Baseline model with random effects added

Fixed Effects: The fixed effects were consistent with these in the Model 1 (Table 5.1 and Table 5.2).

Proportion of variance explained

Analysis of the sources of variation in nestling mass and fledgling survival demonstrated that there were considerable differences between nests: 44% of the variance in mass and 58% of the

variance in survival were attributable to nest ID. While temporal variation across different hatch date intervals and cohorts contributed little to the variance in mass (2% and 1% respectively), and cohort explained less than 6% of the variance in survival, a substantial part of the survival variance - 18% - was associated with hatch date interval. Additive genetic effects explained 13% of the variance in survival and 17% of the variance in mass. Over a third (36%) of the variance in mass remained unexplained (residual variance). Please note that since the residual variance for survival was fixed to 1, the percentages of the explained survival variance accounted for by each component reported above were calculated using the sum of all the non-residual variance components as the denominator.

Covariances and Correlations

The covariances were positive, with the exception of additive genetic covariance. Consequently, the resulting correlations were positive for nest ID, hatch date, and cohort, but negative for additive genetic effect. The correlations between mass and survival ranged from -0.06 to 0.65, and were the strongest due to the effect of differences between hatch date intervals and cohorts (Table 5.2). Figure 5.2 shows how mass and survival changed across the different hatch date intervals, with both being lower earlier in the season and then rising concurrently - generating the positive correlations seen in Table 5.2. The environmental (residual) covariance and correlation were positive.

Statistical significance of the covariances and correlations: the credible intervals (95% CIs) of all covariances spanned 0 and could therefore be considered statistically non-significant. They were, however, quite borderline, with the lower credible intervals just under 0 (Table 5.2). Moreover, there was a slight disagreement between the credible intervals (95% CIs) for the covariances and correlations of hatch date and cohort: the CIs for the correlations did not span zero, implying that these correlations were statistically significant. These differences were due to the skew in the distributions of the correlations: the shortest interval which contains 95% of the probability was chosen for the calculations and therefore the fat-tailed end of the distribution - containing most of the information - was preferred over the long-tailed end. To obtain a further measure of "significance" for the correlations, I calculated the percentage of negative correlation estimates for these random effects: only 2.80% of correlation estimates for hatch date, and only 3.60% of correlation estimates for cohort were negative (Table D.2a).

Given that the CIs of covariances for cohort and hatch date were borderline, with the lower CIs of -0.01 in each case (Table 5.2), the CIs for their correlations did not span zero (Table 5.2), and that less than 5% of the correlation estimates were negative in each case (Table D.2), I conclude that there was support for positive correlations between mass and survival due to hatch date and cohort in this model.

Table 5.2. Model of nestling mass and survival from fledging to independence, with random effects included (Model 2). The fixed effects are: the sex of the nestling, nestling age at measurement, and change in weighing protocol in 1992. The model estimates are based on posterior means, 95% credible intervals (CIs) are given in brackets, and *p*-values are based on *pMCMC*.

	nestling mass		survival from fledging to independence	
Fixed effects	**Estimate (95% CI)**	**P**	**Estimate (95% CI)**	**P**
intercept	-3.24 (-5.78, -0.90)	**0.010**	0.59 (0.14, 1.08)	**0.009**
1992 (1992+, pre-1992)				
pre-1992	0.54 (0.35, 0.74)	<**0.001**		
nestling age	2.00 (1.34, 2.73)	<**0.001**		
nestling age^2	-0.08 (-0.13, -0.03)	<**0.001**		
sex (female, male)				
male	0.15 (0.11, 0.18)	<**0.001**	0.03 (-0.10, 0.16)	0.638
Random effects	**Variance-Covariance-Correlation Matrices (95% CI)**			
	mass		survival	
nest ID				
mass	0.23 (0.21, 0.26)		0.06 (-0.04, 0.15)	
survival	0.04 (-0.02, 0.09)		1.56 (1.14, 1.98)	
hatch date				
mass	0.01 (0.004, 0.03)		0.65 (0.12, 0.99)	
survival	0.05 (-0.01, 0.14)		0.66 (0.17, 1.49)	
cohort				
mass	0.01 (1.62^{-6}, 0.02)		0.58 (0.08, 1.00)	
survival	0.02 (-0.01, 0.05)		0.15 (0.04, 0.31)	
additive genetic effect				
mass	0.09 (0.06, 0.13)		-0.06 (-0.46, 0.32)	
survival	-0.01 (-0.08, 0.05)		0.37 (0.07, 0.75)	
residual variance				
mass	0.19 (0.17, 0.21)		0.10 (-0.02, 0.21)	
survival	0.04 (-0.01, 0.09)		fixed to 1.00	
Sample size	3808			

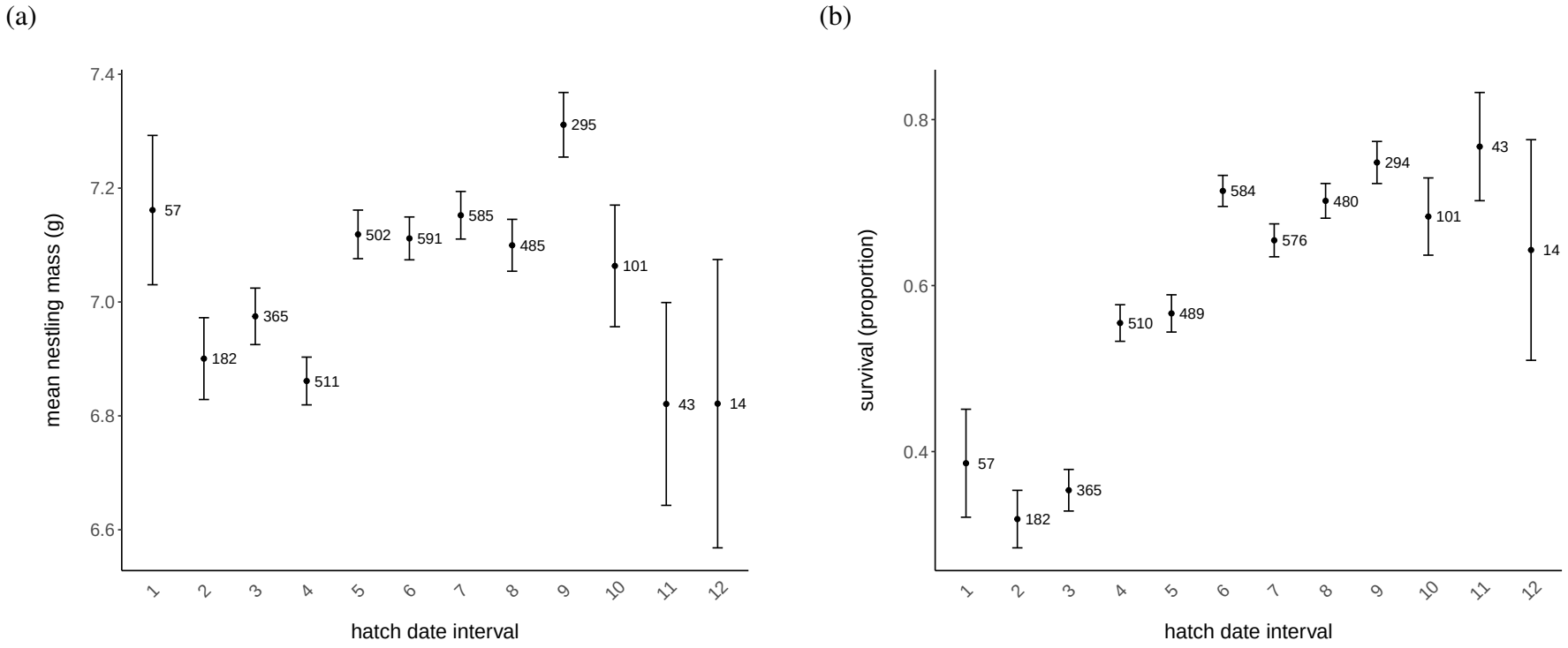

Figure 5.2. The change in mean nestling mass (a) and fledgling survival (b) across hatch date intervals. Each hatch date interval covered a two-week period, starting at the beginning of the breeding season; with interval number one at the end of September, and interval number eight corresponding to the beginning of January. Sample sizes for each hatch date interval are given next to the points.

5.3.3. Model 3. Full model: all fixed effects and all random effects

Fixed Effects

The effects of '1992', nestling age and sex were consistent with those of the baseline model discussed above. Brood size, number of helpers, inbreeding coefficient and within-pair status were fitted as additional fixed effects in the full model. Larger broods had lighter nestlings; nestlings assisted by more helpers were heavier, and the model suggested that individuals assisted by two or more helpers were more likely to survive to independence (Table 5.3). There was also evidence of inbreeding depression in mass, but not in survival. There were no differences in mass or survival between within-pair and extra-pair offspring (Table 5.3).

Proportion of variance explained

Interestingly, the addition of fixed effects did not have a substantial effect on the estimates of variance in either mass or survival - despite the brood size, number of helpers and inbreeding coefficient being statistically significant (Table 5.3). This is illustrated by nearly identical variance component estimates in Table 5.2 and Table 5.3. The proportions of survival variance explained in Model 2 and Model 3 were also nearly identical: the figures for Model 3 are presented below and the figures for both models are listed together in Table D.1 for ease of comparison.

Analysis of the sources of variation in nestling mass and fledgling survival demonstrated that there were considerable differences between nests: 43% of the variance in mass and just under 58% of the variance in survival were attributable to nest ID. While temporal variation across different hatch date intervals and cohorts contributed little to the variance in mass (2% each), and cohort explained just over 5% of the variance in survival, a substantial part of the survival variance - 24% - was associated with hatch date interval.

Additive genetic effects explained under 14% of the variance in survival and 17% of the variance in mass, over and above the effects of inbreeding depression (which was accounted for by fitting inbreeding coefficient (f) in the fixed structure of the model).

Over a third (36%) of the variance in mass remained unexplained (residual variance). Please note that since the residual variance for survival was fixed to 1, the percentages of the explained survival variance accounted for by each component reported above were calculated using the sum of all the non-residual variance components as the denominator.

Covariances and Correlations

As in the previous model, the covariances were positive, with the exception of the additive genetic effect covariance. Consequently, the resulting correlations were positive for nest ID, hatch date, and cohort, but negative for additive genetic effect. The correlations between mass

and survival ranged from -0.09 to 0.70, and were the strongest due to the effect of differences between hatch date intervals and cohorts (Table 5.3). The environmental (residual) covariance and correlation were positive.

Statistical significance of the covariances and correlations: the credible intervals (95% CIs) of all covariances spanned 0 and could therefore be considered statistically non-significant. They were, however, quite borderline, with the lower credible intervals just under 0 (Table 5.3). Again, there was a slight disagreement between the credible intervals (95% CIs) for the covariances and correlations of hatch date and cohort: the CIs for the correlations did not span zero, implying that these correlations were statistically significant. As in Model 2, these differences were due to the skew in the distributions of the correlations: the shortest interval which contains 95% of the probability was chosen for the calculations and therefore the fat-tailed end of the distribution - containing most of the information - was preferred over the long-tailed end. To obtain a further measure of "significance" for the correlations, I calculated the percentage of negative correlation estimates for these random effects: only 1.25% of correlation estimates for hatch date, and only 3.40% of correlation estimates for cohort were negative (Table D.2b).

Given that the CIs of covariances for cohort and hatch date were borderline, with the lower CIs of -0.001 and -0.01 respectively (Table 5.3), the CIs for their correlations did not span zero (Table 5.3), and that less than 5% of the correlation estimates were negative in each case (Table D.2), I conclude that there was support for positive correlations between mass and survival due to hatch date and cohort in this model.

5.4. Discussion

I have shown that mass and survival are correlated in this superb fairy-wren population. Heavier nestlings had a higher probability of surviving to independence (here and Chapter 2), and given that mass was heritable, a response to selection would be expected. However, having explored this relationship further by considering the genetic covariance between the two traits of interest, I found no evidence of any genetic covariance between the trait and fitness. I demonstrated that time plays a crucial role in this association: the correlations between mass and survival were strongest due to the effects of differences between hatch date intervals and cohorts. Below, I first discuss the factors affecting nestling mass and fledgling survival, and then consider the conclusions from the models of the covariance between the two.

Fixed effects associated with mass and survival could be separated into attributes inherent to the individual - such as sex and inbreeding coefficient - and into characteristics of their social environment, such as brood size and number of helpers assisting the brood. The results were consistent with my earlier analysis (Chapter 2). Males were heavier than females and inbred individuals were lighter than outbred individuals, but neither sex nor inbreeding coefficient affected survival. Individuals in nests assisted by more helpers were heavier, as were those with fewer siblings. However, when it came to survival there was only an indication that fledglings

Table 5.3. Full bivariate model of nestling mass and survival from fledging to independence (Model 3). The fixed effects are: the sex of the nestling, nestling age at measurement, and change in weighing protocol in 1992. The model estimates are based on posterior means, 95% credible intervals (CIs) are given in brackets, and *p*-values are based on *pMCMC*.

	nestling mass		survival from fledging to independence	
Fixed effects	**Estimate (95% CI)**	**P**	**Estimate (95% CI)**	**P**
intercept	-3.35 (-6.00, -0.94)	**0.005**	0.77 (0.15, 1.38)	**0.013**
1992 (1992+, pre-1992)				
pre-1992	0.53 (0.33, 0.72)	<**0.001**		
nestling age	2.06 (1.37, 2.81)	<**0.001**		
nestling age^2	-0.08 (-0.13, -0.03)	<**0.001**		
sex (female, male)				
male	0.15 (0.11, 0.19)	<**0.001**	0.04 (-0.10, 0.16)	0.574
brood size	-0.05 (-0.09, -0.01)	**0.022**	-0.10 (-0.23, 0.04)	0.138
helpers $(0, 1, 2+)$				
1 helper	0.08 (0.01, 0.15)	**0.050**	0.19 (-0.04, 0.42)	0.109
2+ helpers	0.15 (0.06, 0.24)	**0.002**	0.27 (0.01, 0.53)	**0.037**
inbreeding coefficient	-3.23 (-5.98, -0.43)	**0.028**	-4.04 (-13.60, 5.48)	0.423
within-pair status (EP, WP)				
WP	0.004 (-0.04, 0.05)	0.855	0.09 (-0.05, 0.24)	0.222
Random effects	**Variance-Covariance-Correlation Matrices (95% CI)**			
	mass		survival	
nest ID				
mass	0.23 (0.20, 0.25)		0.05 (-0.04, 0.15)	
survival	0.03 (-0.03, 0.09)		1.60 (1.12, 2.02)	
hatch date				
mass	0.01 (0.002, 0.03)		0.70 (0.23, 1.00)	
survival	0.07 (-0.01, 0.15)		0.71 (0.17, 1.54)	
cohort				
mass	0.01 (1.06^{-5}, 0.02)		0.56 (0.03, 0.96)	
survival	0.02 (-0.001, 0.05)		0.15 (0.04, 0.31)	
additive genetic effect				
mass	0.09 (0.06, 0.13)		-0.09 (-0.53, 0.28)	
survival	-0.02 (-0.09, 0.05)		0.39 (0.07, 0.84)	
residual variance				
mass	0.19 (0.17, 0.21)		0.11 (-0.01, 0.22)	
survival	0.05 (-0.003, 0.10)		fixed to 1.00	
Sample size	3808			

with two or more helpers may have a higher chance of survival to independence. I have shown earlier (Chapter 2) that within-pair offspring were more likely to be inbred and - when inbred - to have higher inbreeding coefficients than inbred extra-pair offspring; however it appears that there are no further differences, above and beyond the effects of inbreeding depression, in mass and/or survival of within-pair *vs* extra-pair offspring (Table 5.3).

Roughly two-thirds of the overall phenotypic variance in mass (as reported in Table 5.1) was explained by variance in the effects of nest ID, hatch date, cohort and additive genetic effects (Table 5.2). Fixed effects did not account for much of the variance, as demonstrated by the nearly-identical estimates of variance components in the model without (Model 2; Table 5.2) versus that with (Model 3; Table 5.3) fixed effects. Thus, below I use estimates from the full model (Table 5.3) to discuss the covariances and the proportion of variance explained by each of the fitted variance components.

The analysis demonstrated that there were substantial differences between nests: out of all fitted variance components, nest ID explained the most variance in mass (43%) and survival (58%), after accounting for the fixed effects fitted (Table 5.3). Nest ID represents the characteristics of the nest itself (its location, how inconspicuous it is *etc*.), but also any variation associated with the identity of the social pair and identity of the helpers attending the nest. Therefore, the association of nest identity with mass may be linked to the quality of the area the nest is located in and the availability of resources nearby, as well as to the feeding proficiency of the breeding female and dominant male. The variance in nest ID effects may also reflect the influences of social environment - beyond the number of helpers present - on trait variation in this cooperative species. It would be interesting to investigate the territory properties and social environment in more detail, for example through exploration of the habitat and the characteristics of the resident birds, to establish their role in influencing nestling mass by partitioning the variance further.

Mortality at the nestling stage tends to be "all or nothing" for a nest, with either all nestlings being preyed upon when the nest is discovered by a predator, or the nest remaining concealed allowing all nestlings to survive. Thus, survival at the nestling stage is more a function of the nest/brood than of the individual offspring - this is why survival from fledging to independence was considered in this analysis instead of nestling survival. Using fledgling survival should decouple the effects of nest and brood characteristics from the probability of survival. Thus, the variance explained by nest ID is likely linked to the identity of the social pair; however, it is also possible that the nest ID effect accounted for effects of the characteristics of the territory that the nest was located in. For instance, more fledglings may survive in territories with higher levels of cover. In line with this, recent analyses of this population (F. Backhouse, unpubl.) suggest that reproductive success is higher in areas with dense ground cover and sparse canopy than in areas with dense mid-storey cover.

Another notable variance component was that due to hatch date (different fortnights through the breeding season), which explained 24% of the variance in survival to independence. The

conditions that fledglings experience change during the breeding season. For instance, rainfall and temperatures change, and it is likely that the availability of resources fluctuates too - variation in weather and vegetation may influence the abundance of invertebrates [Kwok *et al.*, 2016; Robinson *et al.*, 2018] on which superb fairy-wrens feed. Pressures from predators may change too: predation rates may be higher during periods in which the predators themselves are raising young. For instance, many of the nests in my study population are raided by pied currawongs (A. Cockburn, pers. comm.); currawongs feed on insects during the early stages of their breeding season, but switch to hunting nestlings and juveniles of other bird species once their young hatch [Wood, 1998].

As outlined in the introduction, the breeder's equation predicts an evolutionary response - change in the mean trait value - when there is selection on a heritable trait [Lush, 1937]. However, this relies on several assumptions, which may be broken, and thus the additive genetic covariance between a trait and fitness is a better predictor of a trait's response to selection [Morrissey *et al.*, 2010]. My analysis revealed that additive genetic effects accounted for 17% of the total phenotypic variance in mass and 14% of the total phenotypic variance in survival from fledging to independence. These results provide evidence that these traits are heritable, and that as such they could respond to selection and evolve over time. As illustrated by Figure 5.1, there appeared to be an association between mass and survival at the phenotypic level, giving the impression of selection on body size. Given that mass was heritable, a response to selection may be expected, as both elements of the breeder's equation were satisfied [Lush, 1937].

However, to recap, when an association between two traits, X and Y, is observed, there are three possible scenarios:

(i) X has a causal effect on Y

(ii) Y has a causal effect on X

(iii) a third variable (which may be unmeasured or even unknown) could cause both X and Y to change

Therefore, even though there was a relationship between mass and survival (and total fitness by proxy) in the superb fairy-wren population, it may not necessarily be a causal effect of mass on survival. Out of options (i) and (ii) it is only logically plausible that mass would affect survival in this study system, not vice versa. However, it is possible that a third variable independently affects both traits, creating a statistical association between the two, which looks like selection. I consider both scenarios below.

First, crucially, models that partitioned the genetic covariance found no evidence for additive genetic covariance between the two traits. If mass were having a causal effect on survival, the genetic component of mass would determine survival, and so the genes for mass would also be those for survival (see Figure 1 in Morrissey *et al.* [2010]), creating a genetic covariance. The absence of this covariance here indicates a lack of causality.

Secondly, while statistical analysis alone cannot determine causality, it can indicate cases where there is a lack of causality and it can provide insights into likely mechanisms that may be driving the overall phenotypic association. My analysis suggests that seasonal and annual changes affected both mass and survival in this population, as correlations at the temporal level were the strongest and <5% of the correlation estimates were negative. Considering the relationship between hatch date and the traits of interest: as illustrated by Figure 5.2, nestling mass and fledging survival both were lower earlier in the breeding season and increased over time. Nestlings that hatched later in the breeding season tended to be heavier and also to have higher survival after fledging than nestlings that hatched earlier in the season. Similarly, nestlings tended to be heavier and to have a higher probability of survival in some cohorts than in others (Table 5.3). These changes occurred in both traits of interest across the different hatch date intervals (Figure 5.2). Phenotypes of individuals may be affected by their genotypes, but also by other, environmental, factors - in this case by the timing of hatching and by differences between cohorts (Figure 5.3). As mass and survival were correlated across the breeding season and across years, this generated an association at the phenotypic level, giving the appearance of selection (Figure 5.3). However, this appears to be driven by temporal variation rather than genetics (although note that it is possible that hatch date has a genetic component and thus that it is not a purely non-genetic factor). The results are likely linked to the temporal variation in weather conditions, consequent resource availability and to variable predation pressure experienced by the superb fairy-wrens. This is in agreement with previous work investigating the effects of climate on body size in this population [Kruuk *et al.*, 2015], which showed the importance of high spring rainfall for nestling size.

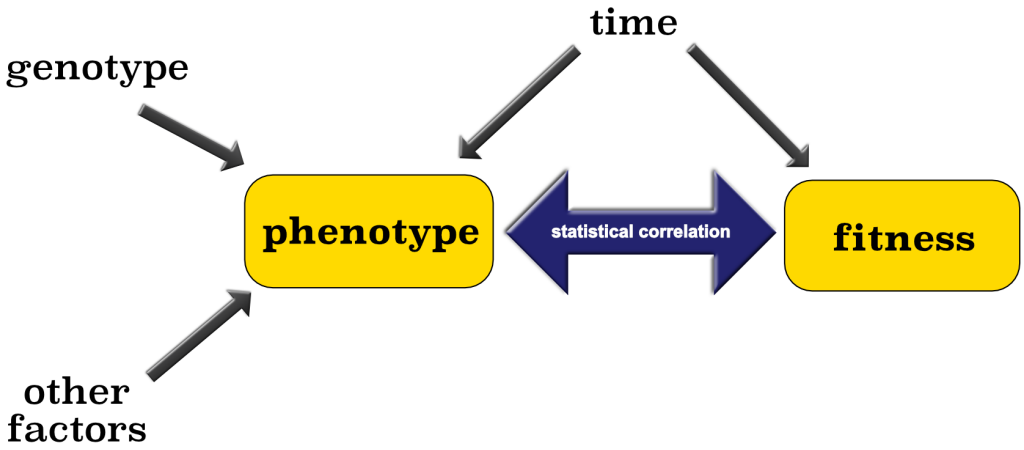

Figure 5.3. Phenotype of an individual is determined by a range of factors, including its genotype. It is possible that a factor, such as time, can be correlated with a phenotypic trait and with fitness. This leads to a statistical correlation between the phenotypic trait and fitness (depicted by double-headed arrow), which makes it appear as if there is a link between the phenotype and fitness (selection). After Kruuk *et al.* [2003].

5.4.1. Conclusions

To summarise, an association between a trait and fitness may give a misleading impression of causal selection: even if traits appear to be under selection, evolutionary change may not occur. This is the case for a large number of traits in natural populations which are expected to evolve. Similarly, in this study system, my analyses indicated a lack of causal effect of nestling mass on survival, despite mass being heritable and heavier individuals having a higher probability of survival. In general, when a lack of response to selection is found, it is difficult to determine the mechanism responsible for the association between the two traits. Merila *et al.* [2001c] discuss studies that fail to detect evolutionary response and stress that "[...] *numerous intensive long-term individual-based studies of natural populations have failed to detect evidence of ongoing evolution, and there are few cases where reported changes can be dissociated from a response to changing environmental conditions, either at the population or the individual level.*" This is a common thread in studies that fail to find predicted evolutionary response despite the appearance of selection and heritability of traits: since they do not detect a genetic link, they - understandably - point to environmental influence. However, given the complexity of natural environments it can be challenging to identify the specific environmental factors responsible for observed change, and in many cases studies will lack data detailed enough to determine which characteristics of the environment are responsible for creating the appearance of selection. Despite that, here I show that covariances at the temporal level are likely candidates explaining this paradox in superb fairy-wrens.

Future work in this study system could investigate the effects of environment (including social environment) in more detail, as well as explore whether the effects of different variance components change across different life-history stages. For instance, nest ID may have less and less of an effect on survival as birds age. Speaking more broadly, a systematic review of studies predicting a response to selection - and failing to find one - and their conclusions, could be quite informative: just how often can the association between a trait and fitness be attributed to specific covariances? And how often the very broad "environmental conditions" are used as an explanation without further investigation? In conclusion, my results demonstrate the importance of explicitly modelling genetic covariances before making predictions about whether a response to the apparent selection should be predicted or not. If such level of scrutiny is not applied, predictions of a response to selection are likely to be inflated.

Chapter 6

Concluding Remarks

6.1. Thesis Overview

Inbreeding, extra-pair reproduction and cooperation have received much research attention over the last few decades. However, it is difficult to obtain the multigenerational genetic information necessary to accurately assess relatedness between individuals. Therefore, it is challenging to study questions at the intersection of these three topics, particularly in natural populations.

In this thesis I investigated context-dependent mate choice and its consequences, focusing on the role of relatedness between individuals and the influence of social environment, concentrating on extra-pair reproduction as the means of mate choice.

I addressed the following questions:

Chapter 2

- What are the patterns of inbreeding and inbreeding depression?
- What is the rate of extra-pair paternity (infidelity) in this population?
- Is the magnitude of inbreeding depression affected by social environment?
- Does female infidelity increase with increasing kinship between social mates?
- Are there consequences of infidelity in terms of offspring inbreeding?
- Are infidelity levels affected by the social environment?

Chapter 3

- What is the distribution of paternity in this population? What are the proportions of within-pair, within-group extra-pair, and extra-group paternity?
- Are a female's infidelity rates affected by the relatedness of her helpers?
- Do helpers of different relatedness affect nest productivity differentially?

Chapter 4

- Do males exercise strategic courtship in order to avoid inbreeding?
- Are males less likely to visit related females than unrelated females?
- Are visitors less likely to display to related females than to unrelated females?

Chapter 5

- Given apparent selection on mass and its heritability, should an evolutionary response in mass be expected in this population?

- How much of the phenotypic association between mass and survival is driven by genetic effects?

- How do differences between nests, time in season and cohort contribute to the variance in mass and survival, and to the covariance between mass and survival?

I addressed these questions using a combination of a long-term dataset and observational data I collected during two field seasons. I utilised mixed effects (animal) models, both univariate and bivariate, under frequentist and Bayesian frameworks.

6.2. Summary of key findings in each chapter

6.2.1. Chapter 2: Inbreeding, inbreeding depression, & infidelity

In Chapter 2, I used 26 years of data to quantify inbreeding, inbreeding depression and infidelity, and to investigate mating strategies, in particular inbreeding avoidance. My analyses showed that the incidence of incestuous pairings ($k_{soc} > 0$) and of inbreeding ($f > 0$) were both rare (10.5% and 5.5% respectively) in this study population. **Assumption 1: Inbreeding leads to inbreeding depression.** I found evidence of inbreeding depression in nestling mass, and that nestling mass affected survival, but this did not translate into evidence for inbreeding depression in fledgling survival. **Assumption 2: Infidelity increases with kinship between social partners.** Increased k_{soc} was associated with a higher frequency of extra-pair offspring, predominantly sired by extra-group males, in the brood. However, the effects of k_{soc} were driven entirely by the complete infidelity observed when mothers were paired to their sons, and there was no indication of an association between social kinship and infidelity in other cases. **Assumption 3: Infidelity reduces inbreeding.** Finally, even though there was no simple relationship between k_{soc} and infidelity, extra-pair offspring were less likely to be inbred than were within-pair offspring, and when inbred, they had lower inbreeding coefficients than within-pair offspring. **Social environment.** While nestlings from broods assisted by helpers were heavier, helpers did not affect the magnitude of inbreeding depression on nestling mass. A higher number of helpers in a group was associated with increased infidelity. However, the social environment did not have an effect on the probability of offspring being inbred.

6.2.2. Chapter 3: Helper relatedness & female infidelity

In the next chapter, I continued using the long-term dataset to investigate associations between the relatedness of helpers and female infidelity. I found that rates of extra-pair paternity increased with the number of unrelated helpers on a territory, but not with the number of helpers related to the breeding female. Rates of extra-pair paternity were 77% when a female had at least one unrelated helper, but only 58% if she had only helper-sons (but no unrelated helpers) and 57%

if she had no helpers. I also showed that the relatedness of helpers was not associated with nest productivity or maternal investment.

6.2.3. Chapter 4: Strategic male courtship & inbreeding avoidance

This chapter complements the previous two: I investigated inbreeding avoidance from the male perspective through exploring strategic male courtship. Additionally, I considered a new social context and focused on males living on different territories than their mothers. In order to carry out this work I collected observational data of males visiting female territories and performing courtship displays over two two-month field seasons. Despite recording evidence of substantial visiting activity, males were significantly less likely to visit the territory of their mother than the territories of other females. I found that females were rarely visited by their sons ($<2\%$ of visits) and that sons never displayed to their mothers.

6.2.4. Chapter 5: Selection on body size

Chapter 5 is tightly linked to Chapter 2, in which I showed that mass was heritable, and that mass and survival were correlated. Given the heritability and appearance of selection, response to selection may be expected. Thus, in the final chapter I examined whether phenotypic association between the two traits of interest, mass and survival, was driven by the additive genetic covariance. I used the long-term data (26 years) and utilised bivariate mixed effects animal models to carry out my analyses. I found that additive genetic effect accounted for 17% of genetic variance in mass and 14% of genetic variance in survival from fledging to independence, but there was no additive genetic covariance between the two traits. I demonstrated that temporal factors - the differences between hatch date intervals and cohorts - were responsible for the correlations between mass and survival at the phenotypic level.

6.3. Implications and future directions

I provided a summary of all the key findings above. Given that Chapters 2-5 have their own discussion sections I would like to avoid unnecessary repetition here. In each of the following sections I address a separate topic. First, I elaborate on the challenges of studying inbreeding depression and mating strategies. Next, I bring together threads from all the chapters and highlight selected results. Finally, I reflect on the avenues that I would have liked to explore and propose potential lines of enquiry for future research.

6.3.1. The challenges of studying inbreeding & mating strategies

In the last few years there has been a renewed interest in exploring mating strategies in relation to inbreeding (hence they are sometimes referred to as 'inbreeding strategies' [Szulkin *et al.*, 2013]). As discussed in Chapters 1 and 2, several research groups have been working on

developing new theoretical models to explore the landscape (e.g. Kokko and Ots [2006]; Lehtonen and Kokko [2015]; Duthie and Reid [2015, 2016]; Duthie *et al.* [2016a]). However, while these provide a great starting point and are valuable, they also use idealised representations of populations. Therefore, conclusions obtained from such models may not necessarily be realistic for each and every study system. Thus, it is beneficial to test these hypotheses empirically, even if a given study system does not conform to all of the assumptions of the idealised model system.

In Chapter 2, I investigated the inbreeding avoidance hypothesis, focusing on infidelity as the means of inbreeding avoidance. As stated there:

> Three assumptions are necessary to support the notion that extra-pair mating occurs to facilitate inbreeding avoidance: (1) that inbreeding depression is present; (2) that infidelity increases with kinship to social mate; and (3) that infidelity reduces the chances of inbreeding.

I have shown that these assumptions ought to be tested concurrently, carefully, and in detail, in order to assess the level of support for infidelity being an inbreeding avoidance mechanism. Additionally, I would also like to highlight that care needs to taken when defining the nature of the assumptions to be tested. For instance, Chapter 2 makes a distinction between what is *required* and what is *sufficient*, particularly when it comes to the presence of inbreeding depression (assumption 1). Existence of inbreeding depression is required for the inbreeding avoidance hypothesis, but it is difficult, if not impossible, to ascertain the precise level of inbreeding depression that would be sufficient to drive the evolution of inbreeding avoidance mechanisms in a given system.

Moreover, whether inbreeding depression is strong enough to expect evolution of inbreeding avoidance mechanisms is arguably a scenario in which the direction of causality is difficult to establish. On one hand, in the presence of strong inbreeding depression, strong selection pressure against inbred individuals could be expected; and thus evolution of inbreeding avoidance mechanisms could be predicted [Pusey, 1987; Blouin and Blouin, 1988; Tregenza and Wedell, 2000]. Otherwise, one could expect selection for inbreeding preference or random mating ('inbreeding tolerance') instead [Szulkin *et al.*, 2013; Duthie and Reid, 2016; Duthie *et al.*, 2016a]. On the other hand, one could also argue that if strong inbreeding depression is not observed, it still could have existed in the past: lack of present inbreeding depression could indicate successful mechanisms reducing inbreeding in the ancestral populations. Therefore, studies investigating these topics may want to specify whether they set out to explore selection pressures leading to the evolution of a behaviour or if they are more interested in the maintenance of said behaviour - the selection pressures involved in both do not need to be the same.

Beyond the theoretical challenges described above, there are also practical difficulties in quantifying inbreeding depression. This is particularly true when attempting to assess the

levels of inbreeding depression in the early life of individuals in wild populations. Presence of inbreeding depression may lead to early offspring mortality, creating a problem of a 'missing fraction' or 'selective disappearance'. This is a situation where inbred individuals die before measurement, leading to underestimation of the level of inbreeding and inbreeding depression, and - in studies investigating infidelity - to overestimation of extra-pair paternity [Reid, 2015; Reid *et al.*, 2015b]. Unfortunately, not all studies investigating inbreeding depression and/or mating strategies address early inbreeding depression in adequate detail, likely due to the difficulties in assessing it. Additionally, results of analyses, and thus the conclusions drawn from them, may also be sensitive to the depth and robustness of the pedigree used [Pemberton, 2004; Harrison *et al.*, 2013]. Pedigrees of open natural populations will always be incomplete, as founding individuals and immigrants have to be considered unrelated to the rest of the studied population.

Working through all these complications I cannot help but think that to really understand these topics we need to clarify our assumptions and definitions, and standardise our approaches. At the moment, comparing different studies is problematic: they may use different types of pedigrees (e.g. social *vs* genetic), use pedigrees of varied completeness, and even employ different definitions of 'inbred' individuals. Moreover, studies generally only test the assumptions selectively - the work presented in Chapter 2 is the only study I am aware of that tests all of the assumptions concurrently - and they may also fail to state explicitly what their assumptions are. Therefore, even though both inbreeding and extra-pair reproduction have been studied for decades and the link between these two is a topic of recent and growing interest, we will struggle to generalise insights gained from separate studies unless we carry out these studies in a consistent manner so that they are comparable.

6.3.2. Links between chapters

Superb fairy-wrens, as cooperative breeders, provided me with an opportunity to explore the influences of social environment on inbreeding and infidelity. I showed that the social environment has more influence on infidelity than on inbreeding: I found no evidence for any effects of helper numbers and characteristics on the occurrence or consequences of inbreeding.

I focus on infidelity in this section, but to quickly recap and bring together results relevant to inbreeding: in Chapter 2, I found an association between mass and survival, as well as inbreeding depression in mass, but not in survival. Lack of support for inbreeding depression in survival was puzzling. Moreover, given that mass was heritable, and that heavier individuals seemed to be more likely to survive, I could expect to see a change in mean mass over time, but that was not the case [Kruuk *et al.*, 2015]. However, in Chapter 5, I showed that there was no genetic covariance between mass and survival and thus a response to selection should not be expected after all. As it turns out, the association between mass and survival in this population can most likely be attributed to temporal factors.

With respect to mating strategies and inbreeding avoidance in particular: females seem to be avoiding inbreeding with their sons, both when they are socially paired (Chapter 2) and when sons act as helpers on the territory (Chapter 3), and males appear to be avoiding inbreeding with their mothers through exercising strategic courtship (Chapter 4). Infidelity is driven by females in this species, and thus it would be easy to view inbreeding avoidance through extra-pair reproduction as female-driven too. However, there is now evidence for both within- and between-group inbreeding avoidance, as well as evidence that both sexes exercise a level of mate choice with regard to relatedness.

In the context of the inbreeding depression levels estimated here, this strict avoidance of mother-son inbreeding is not entirely clear. Initially, there was no evidence in the fairy-wrens of opportunity costs: across all males, within-group success is positively correlated with extra-group success. Furthermore, 31.1% of males socially paired to their mothers sired offspring (all extra-pair) in the same season as being in the mother-son pairs. In comparison, 39.6% of the dominant males that were not in mother-son pairings sired extra-pair offspring in the same season as being dominants. The success of dominants in mother-son pairings is therefore not different than the success of other dominants (Appendix A), suggesting that sons in mother-son pairings do not compensate for the loss of within-pair reproduction through increased extra-pair reproduction. However, my findings in Chapter 4 suggest that there may be some opportunity costs to males. If there is little opportunity cost for a male in mating with a given female, males should attempt to secure as many matings as possible, regardless of any characteristics of the female. On the other hand, if pursuit of mating with one female comes at the cost of mating with another, targeting of particular females will be beneficial - the strategic courtship exercised by males therefore seems to indicate the presence of opportunity costs.

Despite the inbreeding avoidance between first order relatives (mothers and sons), overall infidelity was not explained by either of the hypotheses I tested: it could not be attributed to inbreeding avoidance (Chapter 2) and it was not explained by the constrained female hypothesis either (Chapter 3). Therefore, the hypothesis that females use extra-pair reproduction to realise their mate choice remains the most likely explanation for the extreme levels of infidelity in this population.

Interestingly, infidelity was higher in the presence of helpers (Chapter 2), but as I investigated the influences of social environment on infidelity further, I found that infidelity increased only in the presence of *unrelated* helpers - and even then it was mainly extra-group (Chapter 3). For me, this was the most unexpected result of my thesis and it has puzzled everyone I have discussed it with so far - why does relatedness of helpers matter here? More generally, these results demonstrated that characteristics of conspecifics, such as relatedness to the focal individuals, may influence important aspects of group dynamics in unexpected ways. Therefore, studies investigating the effects of social environment and studies using standard 'indirect genetic effects' models may need to carefully consider whether viewing all individuals as equivalent to each other is not too simplistic.

6.3.3. Unexplored avenues and proposed future research

When I set out to do this PhD I thought that a much larger part of it would be dedicated to investigating the nuances of inbreeding and inbreeding depression. My plans included assessing the severity of inbreeding depression across different life-history stages and exploring sex-specific costs and benefits of inbreeding and mating strategies. However, my early analyses showed that inbreeding events were rarer than expected (Chapter 2). While the large sample size allowed me to quantify the occurrence of inbreeding and assess inbreeding depression in juvenile traits, I could not easily proceed with the rest of my initial plans. However, work is being undertaken to create a new pedigree based on SNP genotypes (Andrew Cockburn, unpubl. data). It would be very interesting to compare the current pedigree to these genomic data (which should give even more accurate estimates of relatedness) and assess the robustness of the results in Chapters 2 and 5. Furthermore, incorporating a few additional years of data and a more accurate genomic pedigree could allow further investigation of the temporal factors creating the association between mass and survival (Chapter 5). This will be explored before turning Chapter 5 into a manuscript. In general, genomic data will allow estimation of actual relationships between individuals and thus enable novel quantitative analyses in this population, including investigation of genomic selection.

The main aspects of the interplay between inbreeding, infidelity and the social environment that I would have loved to investigate revolve around the influences of spatiotemporal factors. For example, in Chapter 2 I showed that overall frequency of inbred individuals increased with certain pedigree restrictions, because of biasing the dataset towards short-dispersing females. Such short-dispersing females may be more likely to encounter relatives - it would be interesting to model that probability explicitly. Furthermore, I also argue in that chapter that the spatiotemporal distribution of potential mates could explain the differences in inbreeding probability and inbreeding level between within-pair and extra-pair offspring. Many studies tend to view all members of the opposite sex present in a population as potential mates, however that approach is not particularly realistic. Both the characteristics and location of individuals will determine their suitability as mates and a spatial analysis would allow their inclusion in the investigation.

I envisaged an analysis specifically taking into account the spatiotemporal distribution of all birds in the population, their key characteristics (such as moult date for males), the relatedness structure of the population and the variation in population size. Ideally, land cover and form would also be taken into account - which would then feed nicely into investigation of environmental factors (habitat characteristics and heterogeneity) suggested in Chapter 4. A combination of mixed effects modelling and spatial modelling with buffering could give a better understanding of the relationship between individuals and their environment. For instance, we could test whether females are less likely to have offspring sired by extra-group males which live in territories that would require the female to travel through a specific type of habitat (e.g. a

valley or a large open lawn). What is even more exciting though, is that given the large volume of data available for this superb fairy-wren population it may be possible to apply machine learning algorithms to investigate state- and context-dependent decision making at the individual level [Frankenhuis *et al.*, 2018, *in press*]. This would allow predicting which males are most likely to sire offspring of a particular female. We finally are at a point where we have statistical tools sophisticated enough to carry out analyses taking into account a larger proportion of the natural world's complexity: such analyses could provide unprecedented levels of insight into mate choice in wild animal populations.

Appendix A

Appendix for Chapter 2: Inbreeding, inbreeding depression and infidelity

A.1. Summary of SI results

These results complement specific sections of Chapter 2, as indicated in bold below.

Section 2.3.1: Pedigree restriction had a considerable effect on the estimates of inbreeding, k_{soc}, and the sample sizes (Table A.1). Most notably, inbreeding rates increased sharply once 3+ known grandparents' restriction was applied. **Section 2.3.2:** There was no indication of early inbreeding depression (Table A.2). The social environment did not mitigate the effects of inbreeding depression on nestling mass or survival (Table A.3). **Section 2.3.3:** Neither the relatedness between social partners (k_{soc}) nor the number of helpers had any effect on the clutch size and/or survival to measurement age (Table A.2). The association between k_{soc} and infidelity was not affected by the social environment (Table A.4). **Section 2.3.4:** I show that my results of within-pair offspring being more likely to be inbred than extra-pair offspring are consistent across (a) pedigree restrictions (Table A.6); (b) classification of "inbred" individuals (Table A.7). Furthermore, social environment was not associated with the probability of offspring being inbred (Table A.8).

A.2. SI Methods

A.2.1. Methods: Paternity assignment

The methods we use to resolve paternity in the superb fairy-wren study population were described in [Double *et al.*, 1997b] and used subsequently for analyses of mating patterns and reproductive success in our study population; see for example Cockburn *et al.* [2003]; Double and Cockburn [2003]; Cockburn *et al.* [2008a]. In brief, the system was based on microsatellite genotyping and involved eight polymorphic microsatellite loci (McyU1-McyU8). Usually in paternity assignment, the probability of excluding a falsely assigned father is calculated in order to assess the utility of the loci. However, this exclusion probability does not account for the population structure present in superb fairy-wrens (clustering of relatives due to male philopatry, presence of reproductively-active male helpers, and male-female reproductive success asymmetry). Because of this, Monte Carlo simulation models that use allele frequencies for multiple loci to estimate the exclusion probabilities when multiple male first-order relatives are considered to be potential sires were developed [Double *et al.*, 1997a]. These models suggested that standard exclusion probability approach was in fact not accurate enough for use in the superb fairy-wren system due to the natal male philopatry and resulting loss of resolution when multiple male relatives are competing for paternity. Therefore, more realistic exclusion probabilities can be obtained by taking into account the characteristics of the system: in this case by assuming that there are close relatives amongst the potential sires and then deciding on the number of loci required to estimate robust paternities, as outlined below.

Subsequent to the initial development of the methods described by Double *et al.* [1997b], we

encountered a deterioration in the utility of some of the microsatellite loci, probably because of the introduction of null alleles. We therefore replaced three loci, discarding McyU1, McyU5 and McyU6 and replacing them with one marker developed for the congeneric *Malurus splendens* (MspU19, [Webster *et al.*, 2004]), one from the confamilial *Stipiturus malachurus* (SmmU7, [Maguire *et al.*, 2006]), and the third from *Terpsiphone mutata* (TmmU6, [Adcock and Mulder, 2002]). The first two of these are moderately variable (approx. 10 alleles in our population), but TmmU6 is hypervariable, with more than 50 alleles.

Our method does not rely on the available statistical programs to ascribe paternity, as the primary problem where ambiguity arises reflects competition between close male relatives such as fathers and sons, or full siblings [Double *et al.*, 1997a], and we also need to allow for moderate mutation rates in key loci [Beck *et al.*, 2003]. We therefore assigned paternity following a series of steps developed specifically to handle these scenarios.

We initially compared young with their mother to identify which alleles in the offspring could have had a paternal origin. Maternity is always known as there is no evidence of egg-dumping in this species. We then examined the social partner of the female to assess his suitability as a sire. Regardless of a match with the social partner, we then tested the ability of all the males known to be alive when the female was fertile to sire the offspring as well. We also included a further set of males defined as 'potentially alive' in this candidate pool, which were birds that have been caught intruding into the study area on courtship displays, and whose territory was not known or monitored so that death date could be precisely determined. We included these extra males in all the years that they might have reasonably been alive: we did not allow the potential sires to exceed 12 years of age, which is the maximum recorded age in the study population.

In the first stage we defined a match at a locus as being within one repeat unit within the microsatellite (2 and 4 bases respectively for dinucleotide and tetranucleotide loci) of the offspring allele or alleles, as most mutations involve a single addition or deletion of a repeat unit [Beck *et al.*, 2003].

In some cases the social partner of the female at the time she had a nest was never caught, and we did not attempt to attribute parentage in these cases, as the male could not be reliably excluded. Subsequent to the study area reaching its full size in 1993 we recorded 10 such offspring in nests attended by 3 different social partners. Just two of these offspring went on to produce young themselves, so the effect of not attributing paternity in these cases on the integrity of the pedigree is limited.

Although in general we unambiguously identified a single best sire, we encountered a number of ambiguities that we resolved with either further analysis or pragmatic rules-of-thumb.

Most commonly (n = 654 offspring), we found that all the males in the comparison set could be reliably excluded as sires (generally $< 75\%$ matches with the offspring's paternally inherited alleles). There is very strong evidence that these offspring were sired by males living outside the

study area. First, they were much more prevalent in territories close to the boundary of the study area. Second, the high success of some of our 'potentially alive' class attests to the importance of out-of-area males: the most successful of these produced 13 and 11 young and 51 and 37 grand-offspring respectively. For the pedigree and calculation of inbreeding coefficients, ignoring the possibility that the offspring had the same out-of-area sire can potentially lead to full sibs being treated as half sibs. It is most parsimonious but by no means inevitable that the offspring in these cases are likely to have just one extra-group sire. We used the program *COLONY* [Jones and Wang, 2010] to identity offspring that were likely to be sired by the same out-of-area male, which is the parsimonious conclusion as clutches are likely to have just one extra-group sire. We could not examine between-year patterns in a single analysis because of computational limitations. We did examine young within subregions of the study area to see whether the same male had been successful in consecutive years. This has thus far led us to recognise 108 unknown males which we treated as sires in the pedigree, which contributed 328 young (50.2% of the original 654). We should note that the *COLONY* test is probably conservative when the number of offspring is just two.

The next problem was the identification of two (or very rarely more) males that matched the young equally according to our initial criteria. These were generally father-son or full-sib pairs. We distinguished between the possibilities according to the following criteria, which are arranged in descending order of frequency. If the matching of one male depended on allowing for mutations but the other did not, we assigned to the male that did not require the assumption of mutation. Second, if one of the competing males exclusively matched some of the other young in the brood or in consecutive broods by the same female, but the second had no unique matches, we assigned paternity to the male with exclusive matches. Third, in a number of cases a male socially paired to his mother matched the offspring, but so did his own extra-group sire (n = 5 offspring from 5 broods by five different mother-offspring pairings). We have strong behavioural evidence that males show no sexual interest in their mothers, so in this case we assigned paternity to extra-group sire. There were also 3 offspring where a son that had been sired by an out-of-area male appeared to have been successful with his mother to which he was socially paired. Because this only occurred in other cases within the study area when the son's sire was alive, we assumed that this was also true in these cases, and we assessed these young to have been produced by extra-group parentage. Finally, for just 3 young we got a match that lived a very great distance from the mother (greater than 6 territories), and a match with a male that lived nearby. In these cases we assigned paternity to the nearby male.

There were 72 offspring that could not easily be distinguished according to these criteria, so we did not assign parentage. Fortunately, 69 of these (96%) produced no young themselves, and hence had no influence on the subsequent structure of the pedigree.

A.2.2. Methods: Pedigree reconstruction and quantifying levels of inbreeding

Using the parentage data, we constructed a pedigree containing individuals sampled between 1988 and 2013. The maximum lineage length in the pedigree was 15 generations. Individuals without known parents were assumed to be unrelated to the rest of the population. Detecting inbreeding requires a minimum knowledge of identity of both parents and at least one grandparent; we therefore only estimated inbreeding coefficients (f) for individuals where the identities of both genetic parents and at least one grandparent were known ($n = 4431$). The depth and accuracy of pedigree data can affect estimates of the prevalence of inbreeding, but restricting a pedigree to individuals with larger amounts of ancestry information inevitably comes at a cost to sample sizes [Walling *et al.*, 2011]. I provide details of the effect of restrictions on the sample sizes and inbreeding rates (Table A.1).

For all social pairs (*i.e.* the dominant male, who was always the oldest male on the territory, and the breeding female), we calculated a kinship coefficient (k_{soc}), defined as the probability that two homologous alleles, one sampled at random from each individual, are identical by descent [Wright, 1922]. Similarly, we calculated a kinship coefficient (k_{ep}) for each female-EP male pair that produced extra-pair offspring. For each offspring, we calculated an inbreeding coefficient (f), defined as the probability that two alleles at any randomly-chosen locus in the individual are identical by descent [Wright, 1922]. Both the pedigree reconstruction and the k and f calculations were carried out using the *pedigreemm R* package [Vazquez *et al.*, 2010]. Throughout, we distinguish between the 'genetic father', to mean the male who sired a particular offspring, and the 'social father', to mean the male who was dominant on the territory at the time that the offspring was hatched, and who may or may not have been the genetic father.

My main dataset therefore contained 4431 individuals with known genetic parents and at least one known grandparent. These came from 1745 broods, involving 579 mothers and 615 genetic fathers, with 1726 unique pairings between mothers and fathers, out of which 1197 involved EP fathers. The 1745 broods came from 863 unique social pairings, involving 579 mothers and 536 social fathers.

The variables fitted in the statistical models varied depending on the model, thus sample sizes for individual models varied and are given alongside the model results. All analyses were carried out in *R* version 3.3.1 [Development Core Team, 2011].

A.2.3. Methods: Inbreeding depression

Inbreeding depression on survival from fledging to independence (41 days).

Since we were interested in individual-level effects of inbreeding on survival, post-fledging survival is more relevant than survival at the nestling stage, where mortality is largely due

to predation of the entire brood. I therefore tested for inbreeding depression in survival to independence. Fledging in this study population usually happens at 12 days and the upper bound of 41 days was chosen as an approximate measure of independence: some young receive provisioning at this age, but the earliest known dispersal of young in my study happened at 41 days post-fledging, showing that the individuals can be independent after that point. During the breeding season we aim to census each bird at least three times each week [Cockburn *et al.*, 2003], thus, "death date" for each individual can be estimated with reasonable accuracy. The effects of survival across other life-history stages (up to recruitment into the breeding population) were also investigated, but since those models gave comparable results they are not presented here.

Model details: To test for inbreeding depression on `survival` from fledging to 41 days, we ran two generalised linear mixed effects animal models using the *MCMCglmm* package [Hadfield, 2010]. I first tested whether inbreeding affected `survival`, and then investigated whether any effect of inbreeding acted through body mass, by including `nestling mass` as a covariate in the analysis to check whether the inbreeding coefficient changed. Survival was modelled as a binary (0/1) response variable, with a binomial error distribution. **Fixed Effects:** `Inbreeding coefficient` (f), the `number of helpers`, `brood size` and an individual's `sex` were fitted as described above (with `nestling mass` as an additional covariate in one of the models). **Random Effects:** `nest ID`, `additive genetic effect`, `cohort` and `hatch date` were fitted, as described above.

I ran the analyses for 3.9×10^6 iterations with a burn-in of 9×10^5 and a thinning interval of 1500, generating 2000 samples from which posterior means and 95% CIs (credible intervals; lower CI, upper CI) were calculated. I used parameter expanded priors (`V = 1, fix = 1, nu = 1, alpha.mu = 0, alpha.V = 1` $\times$ `1000`), following the documentation available for the *MCMCglmm* package, such as the *MCMCglmm* Course Notes by Jarrod Hadfield (see *MCMCglmm*'s CRAN page: https://cran.r-project.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf).

All *MCMCglmm* models: In all models we ensured that these effective sample sizes were above 1000, as effective sample sizes for specific parameters could be lower than 2000 due to presence of autocorrelation. Additionally, any missing values were removed from the fixed predictors in all models, *i.e.* there were no birds of unknown sex or age used in any of the models. Note that the removal of missing values from fixed predictors affected sample sizes used by specific models. No stepwise model selection was used: all fitted parameters were chosen based on their (potential) biological significance.

A.2.4. Methods: Effects of kinship between social partners on infidelity

I fitted binomial generalised linear mixed models using the *R* package *MCMCglmm*, with the `proportion of extra-pair offspring` in a brood (defined by the numbers of extra- *vs* within-pair offspring) as a response variable, and binomial errors; these models were by definition fitted at the level of the brood rather than individual nestlings. **Fixed Effects:** `kinship` (continuous) was fitted to test whether the probability of offspring being sired by an extra-pair male varied with the kinship between the female and her social mate (k_{soc}). The `number of helpers` (0, 1 and 2+) was fitted to test whether the social environment affected the probability of extra-pair reproduction. Additionally, the `mother's age` and the `social father's age` (two level factors: one year old, older) were fitted as fixed effects to account for potential effects of differences in experience. **Random Effects:** `mother ID` and `social father ID` were fitted to account for the multiple observations on specific females and males (social fathers). I also fitted a multi-level factor of `cohort` to account for inter-annual variation.

Model details: iterations = 2.6×10^6; burn-in = 6×10^5; thinning interval = 1000; generating 2000 samples; with parameter expanded priors: `V = 1, n = 0.002`, `nu = 1, alpha.mu = 0, alpha.V = 1 × 1000`.

A.2.5. Methods: Effects of infidelity on inbreeding

Step 1. I fitted a binomial generalised linear mixed model using *MCMCglmm*, with the `inbreeding status` of every offspring as a response (two level factor: inbred *vs* outbred, where an inbred individual was defined as one for which $f > 0$). **Fixed Effects:** I fitted `within-pair status` (whether an offspring was the result of within-pair (WP) or extra-pair (EP) reproduction) as a two-level factor (WP, EP). **Random Effects:** `nest ID` was fitted to account for similarities across multiple offspring from the same brood. I also fitted a multi-level factor of `cohort` to represent inter-annual variation. The model was fitted excluding mother-son pairings to avoid any bias stemming from those special cases.

Step 1 model details: iterations = 3.9×10^6; burn-in = 9×10^5; thinning interval = 1250; generating 2400 samples; with parameter expanded priors: `V = 1, fix = 1`, `nu = 1, alpha.mu = 0, alpha.V = 1 × 1000`.

Step 2. I then tested what determined the magnitude of the inbreeding coefficient amongst those nestlings that were inbred, *i.e.* had $f > 0$. I fitted a linear mixed model using the *MCMCglmm* package, with log-transformed `inbreeding coefficient` (f) as the response variable, and Gaussian errors. I used only the inbred individuals ($f > 0$) in this model. **Fixed Effects:** I fitted `within-pair status` (whether an offspring was the result of within-pair (WP) or extra-pair (EP) reproduction) as a two-level factor (WP, EP). **Random Effects:** As before, `nest ID` and

`cohort` were fitted as random effects. The model was fitted excluding mother-son pairings to avoid any bias stemming from those special cases.

Step 2 model details: iterations = 2.6×10^6; burn-in = 6×10^5; thinning interval = 1000; generating 2000 samples; with parameter expanded priors: `V = 1, n = 0.002`, `nu = 1, alpha.mu = 0, alpha.V = 1 × 1000`.

A.3. Figures fitting

Figures were plotted in *R* using the *ggplot2* package [Wickham, 2009]. Predictions in Figure 2.1a and Figure 2.2 are based on GLMMs fitted in *lme4* [Bates *et al.*, 2015] models equivalent to the *MCMCglmm* models presented in the main text (without the additive genetic effect for Figure 2.1a). This is because we encountered difficulties in obtaining and plotting predictions from *ASReml-R* [Butler *et al.*, 2009] and *MCMCglmm* packages used to run the main models.

A.4. SI further analyses

A.4.1. Further analysis: Pedigree reconstruction and quantifying levels of inbreeding

Pedigree restrictions: effects of grandparents' restrictions

Since the depth and accuracy of the pedigree data can affect estimates of inbreeding, I restricted my dataset accordingly to investigate the impact of the different levels of ancestry information on samples sizes and estimates of the frequency of inbreeding. My main dataset included all individuals with known genetic parents and at least one known grandparent, while further restrictions focused on the number of known grandparents: 2+, 3+ and all 4 grandparents known.

Pedigree restrictions had a considerable effect on the estimates of inbreeding in my population, with increasing level of restrictions resulting in increasing rates of incestuous pairings and of inbreeding (Table A.1). A restriction of requiring 4 grandparents reduced the data sets to 22.7% (broods) and 22.1% (individuals) of their original sizes, but increased the percentage of $k_{soc} > 0$ and $f > 0$ by 18% and 16% respectively. The increased frequency presumably reflects in part the exclusion of pairs/individuals erroneously assigned $k_{soc} = 0$ and $f = 0$ because their ancestry information was not sufficient to identify lower levels of relatedness/inbreeding. However, it is worth noting that the marked increase in inbreeding rate beyond 2+ known grandparents is most likely due to restricting the dataset towards females who have dispersed shorter distances from their natal territory, as my ability to sample all grandparents was often conditioned on these females, and they may be more likely to encounter male relatives as partners than females that disperse over longer distances. In an analysis of a multigenerational red deer pedigree [Walling *et al.*, 2011], similar reductions in sample size and increases in inbreeding with stricter

restrictions were notes, but with a relatively consistent change with increasing restrictions (J. Huisman pers. comm.), rather than the sharp jump observed here from 2+ to 3+ known grandparents.

Table A.1. Pedigree restrictions on number of known grandparents affected not only the sample size, but also the rates of incestuous pairings between social partners ($k_{soc} > 0$) and inbreeding ($f > 0$).

restriction applied	*n* broods	*n* incestuous pairings	incestuous %
1+ known grandparent	1745	183	10.5
2+ known grandparents	1584	171	10.8
3+ known grandparents	533	131	24.6
4 known grandparents	396	113	28.5
restriction applied	*n* individuals	*n* inbred	inbred %
1+ known grandparent	4431	245	5.5
2+ known grandparents	4043	242	6.0
3+ known grandparents	1295	236	18.2
4 known grandparents	977	210	21.5

A.4.2. Further analysis: Inbreeding depression

Inbreeding depression in early survival. Does kinship of social partners affect clutch size or survival to measurement?

Note that this analysis is important for both Section 2, as it gave me an indirect test of inbreeding depression in the wild, and for Section 3, as it allowed me to ensure that my estimates of extra-pair offspring in the brood were not biased. (Note: in response to reviewer comments I also ran several additional analyses, which are summarised in Section 5 below).

It is possible that inbreeding depression in very early survival could bias later estimates of the extent of extra-pair paternity in pairs where social partners are related: in short, if all within-pair offspring died due to inbreeding depression, the brood would appear to be composed of entirely extra-pair offspring and associations of EPP with relatedness of social partners will be overestimated [Reid, 2015; Reid *et al.*, 2015b]. In addition, there is the possibility that females may chose to lay smaller clutches when socially paired to related males [Duthie *et al.*, 2016a]. To assess the likelihood of these scenarios affecting my estimates, we first tested whether kinship between the social partners affected clutch size and/or survival of nestlings to measurement age (survival to measurement age covers both hatchability and subsequent survival of hatched young). I defined clutch size as the number of eggs initially laid, and survival to measurement as the ratio of brood size (the number of offspring in a nest at banding age) to clutch size.

I fitted generalised linear mixed effects models in the *MCMCglmm* package. I fitted (a) `clutch size`, with Gaussian errors, (b) the `proportion of nestlings` making it to

measurement age (brood size / clutch size) with binomial errors, as responses. **Fixed Effects:** A fixed effect of `kinship` (continuous) was fitted to investigate whether either brood or clutch size decreased with k_{soc}. The `number of helpers` (0, 1 and 2+) was also fitted to test whether the social environment affected egg/nestling production. Additionally, `mother's age` and `social father's age` (two level factors: one-year-old, older) were fitted as fixed effects to account for potential differences in experience and/or investment with age. **Random Effects:** `mother ID` and `social father ID` were fitted as random effects to account for the multiple observations on specific females and males (social fathers). I also fitted a multi-level factor of `cohort` to represent inter-annual variation.

I run the analyses for 5.2×10^5 iterations with a burn-in of 1.2×10^5 and a thinning interval of 200, generating 2000 samples from which posterior means and 95% CIs (lower CI, upper CI) were calculated. For all *MCMCglmm* models the effective sample sizes for specific parameters varied due to autocorrelation, but we ensured that they were all above 1000. Similarly, for all *MCMCglmm* models we considered terms to be statistically significant based on 95% CIs not spanning 0 and *pMCMC* values (number of simulations greater or smaller than 0 corrected for number of MCMC samples) calculated by *MCMCglmm* being < 0.05. I run models with parameter expanded priors (`V = 1, n = 0.002, nu = 1, alpha.mu = 0, alpha.V = 1 × 1000`).

Results: Neither the relatedness between social partners (k_{soc}) nor the number of helpers had any effect on the clutch size and/or survival to measurement age (Table A.2). This analysis therefore provided an additional indirect test of inbreeding depression in early survival, and gave no indication of early inbreeding depression.

Table A.2. Effects of kinship between the social male and female and the effects of helpers on (a) clutch size, and (b) hatchability/survival of offspring to measurement age. Output from *MCMCglmm* models: sample sizes are number of broods across 26 cohorts.

	clutch size		**hatchability/survival**	
	(a)		(b)	
Fixed effects	**Posterior mean (95% CI)**	**P**	**Posterior mean (95% CI)**	**P**
intercept	3.11 (3.02, 3.20)	<**0.001**	-0.18 (-0.38, 0.01)	0.078
mother age (1yo, older)				
older	0.13 (0.08, 0.19)	<**0.001**	0.02 (-0.12, 0.17)	0.718
social father age (1yo, older)				
older	-0.02 (-0.09, 0.06)	0.584	0.002 (-0.20, 0.21)	0.973
helpers (0, 1, 2+)				
1 helper	0.01 (-0.04, 0.07)	0.705	-0.01 (-0.17, 0.12)	0.861
2+ helpers	-0.02 (-0.09, 0.05)	0.643	0.01 (-0.15, 0.21)	0.891
kinship	-0.35 (-0.83, 0.15)	0.165	0.13 (-1.10, 1.40)	0.813
Random effects	**Posterior mean (95% CI)**		**Posterior mean (95% CI)**	
mother ID	0.02 (0.01, 0.03)		0.001 (2.62^{-10}, 0.004)	
social father ID	0.002 (4.89^{-11}, 0.01)		0.001 (2.82^{-11}, 0.005)	
cohort	0.01 (0.01, 0.03)		0.001 (8.72^{-11}, 0.005)	
residual	0.22 (0.21, 0.24)		n/a	
Sample size	1740		1699	

Effect of social environment on inbreeding depression

I then tested for inbreeding depression in nestling mass and survival (see the main text for details). Here we present the inbreeding depression models fitted with an interaction between the `inbreeding coefficient` (f) and the `number of helpers`, to test for any effects of social environment and assess whether the presence of helpers could mitigate any effects of inbreeding depression.

To test for inbreeding depression in `nestling mass`, we fitted a linear mixed effects animal model fitted using the *ASReml-R* package, with `nestling mass` as the response, with Gaussian errors. **Fixed Effects:** `inbreeding coefficient` (f), `number of helpers`, `brood size`, `sex` of nestling, `nestling age` at measurement (quadratic function). I also fitted a two level factor '`1992`' (pre-1992, 1992+) to account for the introduction of a new weighing protocol in 1992 [Kruuk *et al.*, 2015]; and an interaction between the `inbreeding coefficient` and the `number of helpers`. **Random Effects:** `nest ID`, an `additive genetic effect` (with covariance structure determined by the pedigree), `cohort`, `hatch date`.

To test for inbreeding depression in `survival` from fledging to 41 days, we fitted generalised linear mixed effects animal models fitted using the *MCMCglmm* package. I ran one model with `nestling mass` fitted as a covariate, as mass may affect survival, and a second model without `nestling mass`; with `survival` as a binary (0/1) response variable, and a binomial error function. Iterations = 3.9×10^6, burn-in = 9×10^5, thinning interval = 1500, samples generated = 2000. Parameter expanded priors: `V = 1, fix = 1, nu = 1, alpha.mu = 0, alpha.V = 1` $\times$ `1000`. **Fixed Effects:** `inbreeding coefficient` (f), `number of helpers`, `brood size` and offspring `sex` (with `nestling mass` as a covariate in the first model). I also fitted an interaction between the `inbreeding coefficient` and the `number of helpers`. **Random Effects:** `nest ID`, `additive genetic effect`, `cohort` and `hatch date`.

Results: The social environment did not mitigate the effects of inbreeding depression on nestling mass or survival (Table A.3).

Table A.3. Test for inbreeding depression and the effects of social environment on the magnitude of inbreeding depression (an interaction between the inbreeding coefficient and number of helpers). Effects of inbreeding coefficient f on (a) nestling mass; and on survival from fledging to 41 days fitted (b) with nestling mass (corrected for change in protocol in 1992 and for nestling age at measurement) included as a covariate, and (c) without nestling mass included. (Note that the precise form of output differs for the *ASReml-R* model in (a) *versus* the *MCMCglmm* models in (b)/(c).)

	nestling mass (g)		**survival from fledging to 41 days**			
	(a)		(b) with mass		(c) without mass	
Fixed effects	**Estimate (SE)**	**P**	**Posterior mean (95% CI)**	**P**	**Posterior mean (95% CI)**	**P**
intercept	-3.83 (1.21)	**0.008**	-0.96 (-3.19, 1.05)	0.370	1.45 (0.21, 2.74)	**0.017**
1992 (1992+, pre-1992)		<**0.001**				
pre-1992	0.62 (0.12)					
nestling age	2.16 (0.35)	<**0.001**				
nestling age^2	-0.09 (0.03)	<**0.001**				
brood size	-0.05 (0.02)	**0.012**	-0.17 (-0.48, 0.12)	0.271	-0.20 (-0.47, 0.11)	0.175
sex (female, male)		<**0.001**				
male	0.15 (0.02)		-0.08 (-0.37, 0.20)	0.611	-0.03 (-0.32, 0.23)	0.866
mass			0.33 (0.11, 0.59)	**0.006**		
helpers $(0, 1, 2+)$		<**0.001**				
1 helper	0.09 (0.04)		0.22 (-0.26, 0.69)	0.384	0.24 (-0.24, 0.74)	0.320
2+ helpers	0.20 (0.04)		0.34 (-0.27, 0.90)	0.253	0.38 (-0.19, 0.95)	0.196
inbreeding coefficient (f)	-3.50 (1.71)	**0.004**	-11.80 (-37.83, 14.50)	0.382	-14.08 (-38.52, 11.30)	0.253
interactions		0.984				
f coef * 1 helper	-0.37 (2.55)		31.70 (-15.61, 76.59)	0.171	32.70 (-11.84, 76.38)	0.132
f coef * 2+ helper	0.44 (5.84)		-48.52 (-151.88, 49.42)	0.332	-41.86 (-148.75, 52.86)	0.403
Random effects	**Variance (SE)**		**Posterior mean (95% CI)**		**Posterior mean (95% CI)**	
nest ID	0.23 (0.01)		6.61 (4.65, 8.83)		6.39 (4.50, 8.30)	
hatch date	0.01 (0.01)		2.70 (0.62, 6.03)		2.72 (0.65, 6.17)	
cohort	0.01 (0.01)		0.31 (6.33^{-6}, 0.75)		0.32 (9.30^{-5}, 0.72)	
additive genetic effect	0.10 (0.02)		1.70 (0.26, 3.60)		1.60 (0.23, 3.22)	
residual variance	0.19 (0.01)		n/a		n/a	
Sample size	4167		3187		3200	

A.4.3. Further analysis: Effects of kinship between social partners on infidelity

Does kinship of social partners affect clutch size or survival to measurement?

Details of the analysis are presented in the above section, as this analysis provided me with an indirect text of inbreeding depression in early survival.

Results: Neither the relatedness between social partners (k_{soc}) nor the number of helpers had any effect on the clutch size and/or survival to measurement age (Table A.2).

Effects of social environment on association between k_{soc} and infidelity

I tested whether patterns of extra-pair paternity were associated with either kinship between social partners (k_{soc}) and/or social environment, specifically the presence of helpers at the nest. Two models were run: (a) using all available data, including the mother-son pairings; and (b) excluding the mother-son pairings. See the main paper for details. Here we present these models fitted with an interaction between `kinship` and the `number of helpers`, to test for the role of social environment.

I fitted a binomial generalised linear mixed model using the *MCMCglmm* package, with the `proportion of extra-pair offspring` in a brood (using the numbers of extra- and within-pair offspring) as a response variable, with binomial errors (iterations = 2.6×10^6; burn-in = 6×10^5; thinning interval = 1000; generating 2000 samples; with parameter expanded priors: `V = 1, n = 0.002, nu = 1, alpha.mu = 0, alpha.V = 1 × 1000`). **Fixed Effects:** `kinship` (k_{soc}), `number of helpers`, `mother's age` and `social father's age`. Interaction between `kinship` and the `number of helpers`. **Random Effects:** `mother ID`, `social father ID`, `cohort`.

Results: There was no significant interaction between k_{soc} and social environment (Table A.4).

A.4.4. Further analysis: Effects of infidelity on inbreeding

Pedigree restrictions: effects of infidelity on the probability of offspring being inbred

I showed above that pedigree restrictions had a substantial effect on inbreeding rates (Table A.1). For completeness, we also present the effects of pedigree restrictions on the numbers and percentages of inbred and outbred within- and extra-pair offspring (Table A.5).

Further, we ran models testing the consequences of extra-pair paternity for the probability of offspring being inbred imposing the above pedigree restrictions, to ensure that the analyses in Table 2.5 were robust to these restrictions.

Table A.4. Effects of kinship between the social male and female and the effects of helpers on the proportion of extra-pair offspring in the brood, including an interaction between k_{soc} and helpers. Models were run (a) on all data, including mother-son pairings; and (b) excluding mother-son pairings and any offspring produced by females socially paired to their sons

	proportion of extra-pair offspring in the brood			
	(a) with mother-son		(b) without mother-son	
Fixed effects	**Posterior mean (95% CI)**	**P**	**Posterior mean (95% CI)**	**P**
intercept	0.46 (0.10, 0.85)	**0.019**	0.49 (0.10, 0.87)	**0.011**
mother age (1yo, older)				
older	0.16 (-0.12, 0.42)	0.243	0.13 (-0.15, 0.39)	0.337
social father age (1yo, older)				
older	-0.16 (-0.56, 0.21)	0.389	-0.14 (-0.52, 0.26)	0.476
helpers (0, 1, 2+)				
1 helper	0.53 (0.24, 0.81)	<**0.001**	0.53 (0.25, 0.82)	<**0.001**
2+ helpers	1.18 (0.80, 1.57)	<**0.001**	1.19 (0.80, 1.56)	<**0.001**
kinship (k_{SOC})	19.11 (12.72, 26.60)	<**0.001**	-4.22 (-18.26, 8.74)	0.530
interactions				
k_{SOC} * 1 helper	0.62 (-11.19, 13.16)	0.951	9.00 (-11.25, 25.94)	0.340
k_{SOC} * 2 helper	1.85 (-17.19, 20.37)	0.939	-7.74 (-49.71, 27.02)	0.682
Random effects	**Posterior mean (95% CI)**		**Posterior mean (95% CI)**	
mother ID	0.73 (0.30, 1.10)		0.69 (0.25, 1.06)	
social father ID	0.72 (0.35, 1.11)		0.73 (0.39, 1.14)	
cohort	0.02 (4.46^{-9}, 0.06)		0.02 (2.09^{-9}, 0.07)	
residual variance	1.88 (1.40, 2.37)		1.82 (1.35, 2.34)	
Sample size	1473		1421	

MCMCglmm truncation of latent variables

Note that all models in this section, regardless of restrictions, were run with an adjusted version of the *MCMCglmm* package allowing for truncation of latent variables: any latent variable that is less than -25 or greater than 25 was rejected at the Metropolis-Hastings stage to avoid under/overflow. This was necessary due to considerable variation in mortality between nests: in most cases either the nest fledged fully or completely failed, and thus there was little partial mortality, which lead to problems with extreme latent variables. I thank Jarrod Hadfield for providing the adjusted version of *MCMCglmm*.

Effects of pedigree restrictions on the probability of offspring being inbred

I fitted a binomial generalised linear mixed models using the *MCMCglmm* package, with the `inbreeding status` of every offspring as a response (two level factor: inbred *vs* outbred, where an inbred individual was defined as one for which $f > 0$). **Fixed Effects:** `within-pair status` (whether an offspring was the result of within-pair (WP) or extra-pair (EP) reproduction). **Random Effects:** `nest ID`, `cohort`. The models were fitted excluding mother-son pairings to avoid any bias stemming from those special cases. Additionally, because the number of helpers affects rates of extra-pair paternity, we did not fit the `number of helpers` in these models, given the potential confounding effects between the two.

Table A.5. Effects of pedigree restrictions on numbers and percentages of inbred and outbred within- and extra-pair offspring. Percentages are presented per row and rounded to 1 decimal place. Any individual with inbreeding coefficient $f > 0$ was classified as inbred.

restriction applied		inbred	outbred	row total
1+ known grandparent	within-pair	130 7.5%	1597 92.5%	1727
	extra-pair	115 4.3%	2589 95.8%	2704
2+ known grandparents	within-pair	127 8.2%	1430 91.8%	1557
	extra-pair	115 4.6%	2371 95.4%	2486
3+ known grandparents	within-pair	127 23.6%	411 76.4%	538
	extra-pair	109 14.4%	648 85.6%	757
4 known grandparents	within-pair	110 27.4%	292 72.6%	402
	extra-pair	100 17.4%	475 82.6%	575

Model settings. **For 1+ known grandparent:** iterations = 3.9×10^6; burn-in = 9×10^5; thinning interval = 1250; generating 2400 samples; **for 2+ known grandparents:** iterations = 5.2×10^6; burn-in = 1.2×10^6; thinning interval = 2000; generating 2000 samples; **for 3+ and 4 known grandparents:** iterations = 2.6×10^6; burn-in = 0.6×10^6; thinning interval = 1000; generating 2000 samples. I used parameter expanded priors: `V = 1, nu = 1, alpha.mu = 0, alpha.V = 1 × 1000`.

Results: Results were consistent across models regardless of the restriction applied: within-pair offspring were more likely to be inbred than extra-pair offspring, even though mother-son pairings were excluded from the datasets (Table A.6).

Table A.6. Pedigree restrictions: effects of within-pair status of an individual (whether it was within-pair, WP, or extra-pair, EP) on the individual's inbreeding status (whether it was inbred, with $f > 0$, or outbred, with $f = 0$), using binomial mixed models run in *MCMCglmm*. The models were run without mother-son pairings.

	inbreeding status of an individual (inbred *vs.* outbred)							
Restriction applied:	**1+ known grandparent**		**2+ known grandparents**		**3+ known grandparents**		**4 known grandparents**	
Fixed effects	**Posterior mean (95% CI)**	**P**	**Posterior mean (95% CI)**	**P**	**Posterior mean (95% CI)**	**P**	**Posterior mean (95% CI)**	**P**
intercept	-9.83 (-10.80, -8.91)	<**0.001**	-9.74 (-10.74, -8.75)	<**0.001**	-6.99 (-8.84, -5.41)	<**0.001**	-6.32 (-8.04, -4.60)	<**0.001**
within-pair status (EP, WP)								
WP	1.36 (0.70, 1.98)	<**0.001**	1.39 (0.75, 2.01)	<**0.001**	1.65 (0.75, 2.54)	<**0.001**	1.88 (0.82, 3.00)	<**0.001**
Random effects	**Posterior mean (95% CI)**		**Posterior mean (95% CI)**		**Posterior mean (95% CI)**		**Posterior mean (95% CI)**	
nest ID	25.12 (21.11, 29.51)		25.34 (21.14, 29.59)		34.71 (25.00, 44.65)		38.48 (26.08, 51.28)	
cohort	2.55 (0.46, 5.23)		2.79 (0.66, 5.55)		10.01 (2.99, 19.32)		9.60 (2.34, 19.30)	
Sample size	4283		3906		1244		943	

Classification by $f \geq 0.0625$ rather than $f > 0$

I treated every individual with inbreeding coefficient $f > 0$ as inbred in the above analyses and in the analyses presented in the main text. However, the large majority of inbred individuals (94.3%) had inbreeding coefficients $f < 0.125$. Therefore, in order to ensure that the many low inbreeding values were not affecting the results disproportionately, we also ran the models on the effects of infidelity on the probability of offspring being inbred using $f = 0.0625$ as a cut-off point: i.e. only individuals with $f \geq 0.0625$ were treated as inbred. As above, we also checked whether restrictions based on different numbers of grandparents affected the results and we did not fit the number of helpers in these models, given the potential confounding effects between the two.

I fitted a binomial generalised linear mixed model using *MCMCglmm*, with the `inbreeding status` of every offspring as a response (inbred *vs* outbred, where an inbred individual was defined as one for which $f \geq 0.0625$). **Fixed Effects:** `within-pair status` (WP *vs* EP). **Random Effects:** `nest ID`, `cohort`. The model was fitted excluding mother-son pairings to avoid any bias stemming from those special cases.

Model settings. **For 1+ known grandparent:** iterations = 3.9×10^6; burn-in = 9×10^5; thinning interval = 1250; generating 2400 samples; **for 2+ known grandparents:** iterations = 5.2×10^6; burn-in = 1.2×10^6; thinning interval = 2000; generating 2000 samples; **for 3+ and 4 known grandparents:** iterations = 2.6×10^6; burn-in = 0.6×10^6; thinning interval = 1000; generating 2000 samples. I used parameter expanded priors: `V = 1, nu = 1, alpha.mu = 0, alpha.V = 1 × 1000`.

Results: Table A.7 shows that the results were nearly identical (compared to Table A.6) whether inbred individuals were classified as $f > 0$ or $f \geq 0625$, regardless of grandparent restriction applied.

Table A.7. Pedigree restrictions: effects of within-pair status of an individual (whether it was within-pair, WP, or extra-pair, EP) on the individual's inbreeding status (whether it was inbred, with $f \geq 0.0625$, or outbred, with $f < 0.0625$), using binomial mixed models run in *MCMCglmm*. The models were run without mother-son pairings.

	inbreeding status of an individual (inbred *vs.* outbred)							
Restriction applied:	**1+ known grandparent**		**2+ known grandparents**		**3+ known grandparents**		**4 known grandparents**	
Fixed effects	**Posterior mean (95% CI)**	**P**	**Posterior mean (95% CI)**	**P**	**Posterior mean (95% CI)**	**P**	**Posterior mean (95% CI)**	**P**
intercept	-11.18 (-12.18, -10.09)	<**0.001**	-11.10 (-12.15, -9.95)	<**0.001**	-10.57 (-12.13, -9.05)	<**0.001**	-10.34 (-11.99, -8.65)	<**0.001**
within-pair status (EP, WP)								
WP	1.21 (0.27, 2.18)	**0.016**	1.10 (0.16, 2.18)	**0.035**	1.99 (0.69, 3.53)	**0.006**	1.85 (0.33, 3.45)	**0.017**
Random effects	**Posterior mean (95% CI)**		**Posterior mean (95% CI)**		**Posterior mean (95% CI)**		**Posterior mean (95% CI)**	
nest ID	17.16 (13.56, 20.93)		17.26 (13.33, 21.32)		23.06 (16.45, 30.48)		24.15 (16.51, 33.04)	
cohort	1.06 (1.32^{-6}, 3.29)		1.06 (3.72^{-7}, 3.18)		2.28 (1.64^{-6}, 7.21)		2.29 (3.44^{-9}, 7.55)	
Sample size	4283		3906		1244		943	

Effects of social environment on the probability of offspring being inbred

Finally, we also fitted models looking at the probability of offspring being inbred as a response with the number of helpers as a sole fixed effect in order to test for the influence of the social environment. I fitted two binomial generalised linear mixed models using *MCMCglmm* two models: (a) inbred $f > 0$ and (b) inbred $f \geq 0.0625$. `Inbreeding status` of every offspring was fitted as a response (inbred *vs* outbred). **Fixed Effects:** `number of helpers`. **Random Effects:** `nest ID`, `cohort`. The models were fitted excluding mother-son pairings to avoid any bias stemming from those special cases.

Model settings: (a) iterations = 2.6×10^6; burn-in = 0.6×10^6; thinning interval = 1000; generating 2000 samples; (b) iterations = 3.9×10^6; burn-in = 9×10^5; thinning interval = 1250; generating 2400 samples. I used parameter expanded priors: `V = 1, nu = 1, alpha.mu = 0, alpha.V = 1 × 1000`.

Results: Helpers did not have an effect on the offspring's probability of being inbred (Table A.8).

Table A.8. Effects of social environment - number of helpers - on the probability of offspring being inbred. Two different ways of classifying "inbred" individuals were used (a) inbred were $f > 0$, (b) inbred were $f \geq 0.0625$. These binomial generalized linear mixed models were run in *MCMCglmm*.

	inbreeding status of an individual (inbred *vs* outbred)			
	(a) outbred $f = 0$		(b) outbred $f < 0.0625$	
Fixed effects	**Posterior mean (95% CI)**	**P**	**Posterior mean (95% CI)**	**P**
intercept	-10.58(-11.67, -9.46)	<**0.001**	-10.57 (-11.63, -9.44)	<**0.001**
helpers (0, 1, 2+)				
1 helper	0.12 (-1.35, 1.41)	0.845	0.10 (-1.22, 1.51)	0.879
2+ helpers	-1.06 (-2.97, 0.62)	0.262	-1.11 (-2.87, 0.62)	0.212
Random effects	**Posterior mean (95% CI)**		**Posterior mean (95% CI)**	
nest ID	17.60 (13.84, 20.96)		17.64 (13.95, 21.47)	
cohort	0.98 (1.85^{-6}, 3.04)		1.01 (5.03^{-7}, 3.05)	
Sample size	4283		4283	

A.4.5. Additional analyses following review in *Evolution*

In response to reviewer requests we ran several additional analyses. These involved:

(i) A test of whether **inbred offspring are differentially cared for**, which could offset the negative effects of such inbred offspring having lower nestling mass, thus explaining no evidence of inbreeding depression in fledgling survival to independence despite reduced survival of lighter offspring. I assumed that parents cannot distinguish between inbred and outbred nestlings within a brood and therefore all offspring in a brood including inbred nestlings would

have to receive increased parental care. If true, outbred offspring raised in the nest with inbred offspring should have higher mass than outbred offspring in 'fully-outbred' nests. I ran a model in *ASReml-R*, with the `nestling mass` of outbred offspring as a response (Gaussian errors) and '`sharing the nest with inbred offspring`' (yes/no) as a fixed effect. Other fixed and random effects fitted followed the model in Table 2.2a (but of course without fitting the `inbreeding coefficient`, as all used offspring were outbred). **Results:** I found no effect of sharing the nest with inbred offspring on outbred nestlings' mass ($pMCMC = 0.623$), indicating that parents do not adjust their investment in the presence of inbred offspring.

(ii) A test of whether the **inbreeding coefficient of offspring is affected by kinship** between the social partners as the relationship between clutch mortality (selective disappearance) and kinship (k_{soc}) could be affected if k_{soc} causes a female to differentially mate with extra-pair males of specific relatedness. I ran the model in *MCMCglmm*, with Gaussian errors, log-transformed response, and parameter expanded priors. The traces, autocorrelation, and effective sample sizes were satisfactory, although posterior means appeared rather high. **Results:** Kinship was not associated with the inbreeding coefficient values of offspring ($pMCMC = 0.958$).

(iii) A test of whether the **number of extra-pair sires per brood increased with kinship** between the social partners, as the relationship between clutch mortality (selective disappearance) and k_{soc} could be affected if k_{soc} causes a female to change the number of extra-pair sires per clutch. I ran a model in *MCMCglmm*, with Poisson errors, the `number of extra-pair sires per brood` as a response, the `kinship` as the sole fixed effect, and the `mother ID`, `social father ID` and `cohort` as random effects. I ran the model both with and without the mother-son pairings. **Results:** The number of extra-pair sires in a brood (1, 2, 3 or 4 - only one case of 4) was not associated with kinship between the social partners ($pMCMC = 0.883$).

(iv) **Opportunity cost.** Reviewer wondered whether the lack of mother-son mating between mother-son social pairs is linked to such matings affecting the son's opportunity to mate with other females (extra-pair). I compared the EP success of dominants in mother-son pairs and other dominants. **Results:** There was no evidence that being paired to mothers affected male's EP success compared to other dominant males (Chi-squared test, $p = 0.324$).

(v) The reviewer also enquired about the effects of grandparents' restrictions on the inbreeding depression and the severity of inbreeding depression. I provide an additional table (see below) showing predicted nestling mass for different grandparent restrictions, across a range of inbreeding coefficients. It clearly shows that the magnitude of the inbreeding depression in mass does not change dramatically across the restrictions, at the same time drawing attention to 1+/2+ and 3+/4 restrictions encompassing a different set of individuals (Table A.9), which, together with the sample-size trade-off, was part of the reason we decided to present results for the 'at least one grandparent restriction.

Table A.9. Predicted nestling mass (g) for increasing levels of inbreeding (standard errors in brackets). Predictions were obtained from the *ASReml-R* models by averaging the fixed effects with the `predict.asreml` function.

	restriction level - number of known grandparents			
inbreeding coefficient (f)	1+	2+	3+	4
0.00000	7.26 (0.07)	7.25 (0.08)	7.13 (0.20)	6.87 (0.24)
0.01560	7.20 (0.08)	7.20 (0.08)	7.08 (0.20)	6.83 (0.24)
0.03125	7.15 (0.08)	7.14 (0.09)	7.03 (0.20)	6.78 (0.24)
0.06250	7.03 (0.11)	7.03 (0.11)	6.93 (0.22)	6.69 (0.26)
0.12500	6.81 (0.17)	6.81 (0.18)	6.74 (0.28)	6.51 (0.32)
0.25000	6.35 (0.32)	6.37 (0.34)	6.36 (0.44)	6.15 (0.48)
% difference (mass for $f = 0$ *vs* $f = 0.25$)	12.5%	12.1%	10.8%	10.5%
total sample size per restriction	4167	3805	1222	922

Appendix B

Appendix for Chapter 3: Unexpected effects of helper relatedness on infidelity

B.1. Additional information for models presented in the main text

This section includes additional information on *MCMCglmm* model specification (iterations, burn-in, thinning interval) and details of parameter expanded priors used. It also includes the details for an analysis of maternal investment through investigation of effects of different helper relatedness levels on clutch size (Table B.1); and an alternative formulation of nestling mass model and its results (Table B.2).

B.1.1. Effects of helper relatedness on female infidelity

I fitted binomial generalised linear mixed effects models using the R package *MCMCglmm* [Hadfield, 2010], with response variables of (a) the `proportion of extra-pair` offspring in a brood (defined by the numbers of extra- *vs* within-**pair** offspring); and (b) the `proportion of extra-group` offspring in a brood (defined by the numbers of extra- *vs* within-**group** offspring). In both models the fixed and random structures were the same. **Fixed Effects:** For each brood, the `number of helper-sons` (0-4) and `number of unrelated helpers` (0-4) were fitted as the main variables of interest. I also fitted `mother's age` and the age of the dominant male on the territory (`social father's age`), both as a two-level factor of one-year-old *vs* older, to account for different levels of experience. **Random Effects:** `mother ID` and `social father ID` were fitted to account for multiple observations. `Cohort` was fitted as a multilevel factor to account for year-to-year variation (1988-2013; each cohort consists of nestlings from one breeding season, *i.e* from August till March).

I ran these analyses for 2.6×10^6 iterations with a burn-in of 0.6×10^6 and a thinning interval of 1000, generating 2000 samples from which posterior means and 95% CIs (credible intervals; lower CI, upper CI) were calculated.

I used parameter expanded priors (set to: `V = 1, n = 0.002`, `nu = 1, alpha.mu = 0`, `alpha.V = 1 × 1000`). I followed the documentation available for the *MCMCglmm* package; the *MCMCglmm* Course Notes by Jarrod Hadfield can be found online (https://cran.rproject.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf).

B.1.2. Effects of helper relatedness on clutch size

I assessed whether different levels of helper relatedness had implications for maternal investment by modelling clutch size.

I fitted a linear mixed effects model in *ASReml-R*, with `clutch size` as the response variable, and Gaussian errors. **Fixed Effects:** As above, I fitted fixed effects of the

`number of helper-sons` (0-4) and the `number of unrelated helpers` (0-4), and `mother's age` and `social father's age` (one-year-old *vs* older). **Random Effects:** `mother ID`, `social father ID` and `cohort` were fitted as above.

Results: Clutch size was not affected by the number of either helper-sons or unrelated helpers, or by the age of social father (Table B.1). Older mothers laid larger clutches than one-year-old females (Table B.1).

Table B.1. Effects of the numbers of helper-sons and unrelated helpers on clutch size. These analyses were carried out at the brood level, in *ASReml-R*, with standard errors reported in brackets.

	clutch size	
Fixed effects	**Estimate (SE)**	**P**
intercept	3.12 (0.05)	<**0.001**
mother age (1yo, older)		<**0.001**
older	0.13 (0.03)	
social father age (1yo, older)		0.570
older	-0.02 (0.04)	
number of helper-sons	0.03 (0.02)	0.102
number of unrelated helpers	-0.01 (0.02)	0.661
Random effects	**Variance (SE)**	
mother ID	0.02 (0.01)	
social father ID	<0.001 (<0.001)	
cohort	0.01 (0.01)	
residual	0.23 (0.01)	
Sample size	1897	

B.1.3. Effects of helper relatedness on nest productivity

Early survival

I fitted a generalised linear mixed effects model in *MCMCglmm*, with binomial errors and the `proportion of offspring surviving to measurement age` (defined using brood size and clutch size) as the response. **Fixed Effects:** Again, `number of helper-sons` (0-4) and `number of unrelated helpers` (0-4) were fitted to test for the impact of relatedness; as well as `mother's age` and `social father's age` (one-year-old *vs* older). **Random Effects:** I fitted `mother ID`, `social father ID` and `cohort` as above.

Model details: iterations = 2.6×10^5; burn-in = 0.6×10^5; thinning interval = 100; generating 2000 samples; with parameter expanded priors: `V = 1, fix = 1`,

`nu = 1, alpha.mu = 0, alpha.V = 1 × 1000`.

Nestling mass - additional model

I have fitted a model where the numbers of helper-sons and unrelated helpers have been fitted as fixed effects to establish whether nestling mass was associated with helper-relatedness (see the main text). That model indicated that both the increase in numbers of helper-sons and unrelated helpers were associated with increased nestling mass - and the mass increased to the same degree. Therefore, I also fitted an additional model with the `total number of helpers` as a three-level factor (0, 1 and 2+) and `helper relatedness` ('mixed relatedness' helper groups, all helper-sons, all unrelated helpers) instead of the numbers of unrelated helpers and helper-sons; with the rest of the fixed and random structures identical to these described in the main model (see below for details).

Model: I fitted a linear mixed effect model in *ASReml-R*, with `nestling mass` as the response variable, and Gaussian errors. **Fixed Effects:** The total `number of helpers` (three-level factor: 0, 1 and 2+) and `helper relatedness` (three-level factor: mixed relatedness helpers, all helper-sons, all unrelated helpers) were fitted to ensure that there were no differences in the effects of helpers of different relatedness on nestling mass. `Inbreeding coefficient` of each offspring was fitted to account for inbreeding depression in nestling mass (effects of inbreeding depression on nestling mass were established in Chapter 2). `Brood size` (continuous) was fitted to account for the differences in the amount of competition for resources provided to each offspring. `Sex` of the nestling (female *vs* male) was fitted to account for size differences between the sexes. `Nestling age` at measurement was fitted as a quadratic function to account for the fact that nestlings were weighed at different ages (practical fieldwork restrictions); and a two level factor '`1992`' (pre-1992 *vs* 1992+) was fitted to account for weighing protocol change in 1992 [Kruuk *et al.*, 2015]. **Random Effects:** I fitted `nest ID` to account for similarities between siblings from the same brood; a multi-level factor of `hatch date` to account for intra-annual variation.

Results: This model confirmed that the increase in the nestling mass was associated with the presence of helpers rather than their relatedness to the breeding female (Table B.2).

Table B.2. Effects of the number of helpers and helper relatedness on the nestling mass. This analysis was carried out at a level of individual offspring, with the model ran in *ASReml-R*.

	nestling mass	
Fixed effects	**Estimate (SE)**	**P**
intercept	-3.69 (1.13)	**0.002**
1992 (1992+, pre-1992)		<**0.001**
pre-1992	0.58 (0.10)	
nestling age	2.10 (0.33)	<**0.001**
nestling age^2	-0.08 (0.02)	<**0.001**
brood size	-0.05 (0.02)	**0.020**
sex (female, male)		<**0.001**
male	0.15 (0.02)	
inbreeding coefficient	-3.10 (1.19)	**0.009**
number of helpers (0, 1, 2+)		<**0.001**
1 helper	0.18 (0.09)	
2+ helpers	0.27 (0.07)	
helper relatedness (mixed, ...)		0.665
all helper-sons	-0.08 (0.08)	
all unrelated helpers	-0.07 (0.08)	
Random effects	**Variance (SE)**	
nest ID	0.27 (0.01)	
hatch date	0.01 (0.01)	
cohort	0.01 (0.01)	
residual variance	0.24 (0.01)	
Sample size	4908	

B.2. Replication study

In this section I include the details of my replication study: I reproduced the model looking into the effects of helper relatedness on female infidelity across multiple subsets of the dataset to ensure that the results were repeatable.

I split the dataset into two groups, allocating 50% of data (964 and 965 broods) to each group at random and repeating this process five times, resulting in ten subsets. I then ran two models, one for the proportion EP offspring and one for the proportion of EG offspring in the brood (see below) on each of these ten subsets.

I fitted binomial generalised linear mixed effects models using the *R* package *MCMCglmm* [Hadfield, 2010], (a) with the `proportion of extra-pair` offspring in a brood (defined by the numbers of extra- *vs* within-pair offspring); and (b) with the `proportion of extra-group` offspring in a brood (defined by the numbers of extra- *vs* within-**group** offspring); as response variables, and with binomial errors. In both models the fixed and random structures were the same. **Fixed Effects:** `Number of helper-sons` (0-4) and `number of unrelated helpers` (0-4) were fitted to test for the impact of relatedness; `mother's age` and the `social father's age` (two level factor: one-year-old *vs* older) were fitted to account for different levels of experience. **Random Effects:** `mother ID` and `social father ID` were fitted to account for multiple observations. `Cohort` was fitted as a multilevel factor to account for year-to-year variation (1988-2013, each cohort consists of nestlings from one breeding season, *i.e* from August till March).

Model details: iterations = 2.6×10^5; burn-in = 0.6×10^5; thinning interval = 100; generating 2000 samples; with parameter expanded priors: `V = 1, n = 0.002, nu = 1`, `alpha.mu = 0, alpha.V = 1` $\times$ `1000`.

Results: Both extra-pair (EP) paternity and extra-group (EG) paternity consistently increased in the presence of the unrelated helpers, but not in the presence of the helper-sons. The other variables were less consistent across the models (Figure B.1).

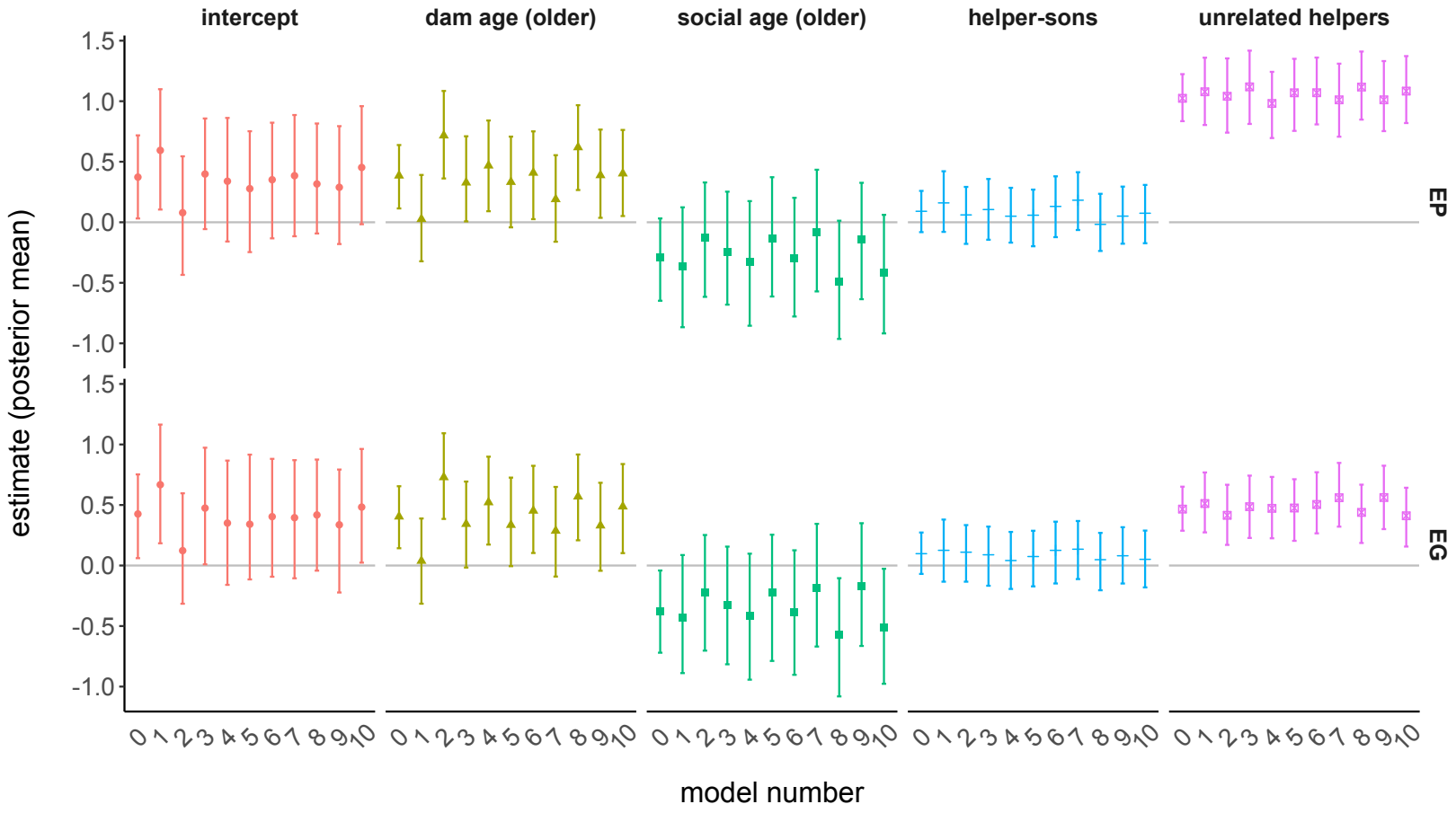

Figure B.1. Replication of the model investigating association between helper relatedness and proportion of offspring resulting from infidelity (extra-pair = EP, and extra-group = EG). Models were run in *MCMCglmm* and the error bars represent 95% credible intervals. The estimates from the 'original' model that used all the data are added for comparison (model number = 0).

B.3. Dominant male's sperm characteristics

Males with sperm with a longer flagellum and relatively shorter heads are more successful at preventing cuckoldry [Calhim *et al.*, 2011]. Given the results of helper relatedness (unrelated helpers increase extra-group paternity) I decided to run *post hoc* analyses looking into 'fertility'-related characteristics of the dominant males, specifically their flagellum length and flagellum/head ratio of their sperm.

I fitted linear mixed effects models using the *R* package *MCMCglmm*, (a) with the `sperm flagellum length`; and (b) with the `relative sperm head length` (defined as flagellum length/head length); as response variables, and with Gaussian errors. In both models the fixed and random structures were the same. **Fixed Effects:** `Number of helper-sons` (0-4) and `number of unrelated helpers` (0-4) were fitted to test for the impact of relatedness; and the `male's age` (two level factor: one-year-old *vs* older) was fitted to control for changes in sperm characteristics with age. **Random Effects:** `male ID` was fitted to account for multiple observations.

Model details: iterations = 3.9×10^5; burn-in = 0.9×10^5; thinning interval = 100; generating 2000 samples; with parameter expanded priors: `V = 1, n = 0.002`, `nu = 1, alpha.mu = 0, alpha.V = 1 × 1000`.

Results: The sperm characteristics of the dominant males were not associated with the relatedness of helpers in the group (Table B.3).

Table B.3. Analysis of the impact of the numbers of helper-sons and unrelated helpers on the sperm characteristics of dominant males, (a) flagellum length, and (b) relative head length. Models ran in *MCMCglmm*.

	sperm characteristics of the dominant male			
	(a)		**(b)**	
	flagellum length		**relative head length**	
Fixed effects	**Estimate (95% CI)**	**P**	**Estimate (95% CI)**	**P**
intercept	66.32 (65.34, 67.20)	<**0.001**	4.44 (4.31, 4.55)	<**0.001**
male age (1yo, older)				
older	-0.42 (-1.58, 0.76)	0.464	0.10 (-0.05, 0.26)	0.217
number of helper-sons	0.27 (-0.53, 1.15)	0.525	-0.06 (-0.16, 0.06)	0.307
number of unrelated helpers	0.08 (-1.15, 1.33)	0.903	0.08 (-0.08, 0.25)	0.362
Random effects	**Variance (95% CI)**		**Variance (95% CI)**	
male ID	2.04 (<0.001, 3.58)		0.03 (<0.001, 0.06)	
residual variance	0.58 (<0.001, 2.51)		0.02 (<0.001, 0.05)	
Sample size	41		41	

Appendix C

Appendix for Chapter 4: Male mate choice and inbreeding avoidance

C.1. Additional statistical models

I fitted generalised linear mixed models, modelling `visits` as a binary response. The models were fitted using all data (with the main model presented in the body of Chapter 4), as well as only using the watches during which at least one visitor was observed (see below, Table C.2).

For each candidate male I scored whether or not he visited the focal female during each watch (total of 3379 possible visits). All models had the same fixed and random structures, as detailed below. Data with missing fixed effects were removed from the models and the numbers of 'observations' used are given alongside model results in the tables. Non-independence of data was taken into account in the random structure of the models.

Fixed Effects: I fitted whether the focal female was the candidate male's mother (`candidate visiting his mother?` ; two-level factor: no, yes), to test whether males avoid visiting or displaying to their mothers; the `candidate's age` and `focal female's age` (both as two-level factors: one-year-old *vs* older) to account for any differences in experience and/or attractiveness; `distance between the candidate & female` - the distance between the centre of the candidate male's territory and the focal female's territory (continuous, rounded to the nearest metre) to account for how far each male had to travel to visit a specific female; `candidate's moult date` , as males only display once they complete the moult; `candidate's social status` (two-level factor: dominant, helper) to account for potential differences stemming from the social position within a group; the `field season year` in which the watch was carried out (two-level factor: 2015, 2016); and the `number of helpers` (continuous: 0, 1, 2) on the focal female's territory, as larger groups could potentially be more vigilant against visitors. **Random Effects:** The date of trial was fitted (`trial date`), as multiple watches were carried out each day over a total of 70 days; the focal `female's ID` was included to account for each female being observed multiple times; and the `candidate's ID` was fitted, as each male could visit multiple females and/or visit multiple times.

Additionally, as the effects of `candidate's moult date` could be confounded with the effects of `candidate's age` , I fitted a model dropping `candidate's age` from the fixed effects (data for all trials were used; Table C.1)

The models were fitted using the R package *MCMCglmm* [Hadfield, 2010], with binomial error distributions. I aimed for effective sample sizes of 2000, ensuring that they were always above 1000. Variables with missing fixed effects were removed. Terms were considered statistically significant when the 95% credible intervals (CIs) did not span zero and when *pMCMC* values < 0.05 ($pMCMC =$ the number of simulations in which the parameter estimate was greater or less than zero, corrected for number of *MCMC* samples). Parameter expanded priors were used (`V = 1, fix = 1, nu = 1, alpha.mu = 0, alpha.V = 1 × 1000`), and the analyses were run for 1.3×10^6 iterations with a burn-in of 3×10^5 and a thinning interval of 500.

Table C.1. What affects the probability of a female being visited by a candidate male? This model is equivalent of model presented in Table 4.1, but was fitted *without* the candidate's age; fitted in *MCMCglmm* using data from all trials. 'Number of helpers' is the number of helpers residing on the focal female's territory at the time of the potential visit.

	visited (no, yes)	
Fixed effects	**Estimate (95% CI)**	**P**
intercept	213.57 (63.47, 366.00)	**0.005**
number of helpers	-0.26 (-0.70, 0.15)	0.197
field season year (2015, 2016)		
2016	3.65 (1.50, 7.90)	**0.003**
candidate's social status (dominant, helper)		
helper	-0.47 (-1.12, 0.18)	0.179
focal female's age (1yo, older)		
older	-0.49 (-1.39, 0.32)	0.241
candidate's moult date	-0.01 (-0.02, 0.004)	**0.005**
distance between the candidate & female	-0.02 (-0.03, -0.02)	**<0.001**
candidate visiting his mother? (no, yes)		
yes	-1.90 (-3.04, -0.74)	**0.001**
Random effects	**Variance (95% CI)**	
trial date	0.20 (<0.001, 0.44)	
female ID	0.53 (0.08, 1.16)	
candidate ID	0.66 (0.24, 1.24)	
residual	n/a	
Sample size	3379	

Table C.2. What affects the probability of a female being visited by a candidate male, considering only watches during which at least one visitor was observed? The model was fitted in *MCMCglmm*; using 139 watches of 18 focal females, with a total of 2076 possible visits. 'Number of helpers' is the number of helpers residing on the focal female's territory at the time of the potential visit. Estimates with $p < 0.05$ are in bold.

	visited (no, yes)	
Fixed effects	**Estimate (95% CI)**	**P**
intercept	186.49 (5.13, 354.71)	**0.038**
number of helpers	-0.07 (-0.40, 0.25)	0.691
field season year (2015, 2016)		
2016	3.91 (0.03, 7.48)	**0.041**
candidate's social status (dominant, helper)		
helper	-0.19 (-0.91, 0.59)	0.601
focal female's age (1yo, older)		
older	0.04 (-0.60, 0.60)	0.887
candidate's moult date	-0.01 (-0.02, -0.001)	**0.038**
distance between the candidate & female	-0.03 (-0.03, -0.02)	<**0.001**
candidate's age (1yo, older)		
older	0.96 (0.001, 1.79)	**0.027**
candidate visiting his mother? (no, yes)		
yes	-1.99 (-3.18, -0.76)	<**0.001**
Random effects	**Variance (95% CI)**	
trial date	0.04 (<0.001, 0.13)	
female ID	0.06 (<0.001, 0.22)	
candidate ID	0.80 (0.30, 1.46)	
residual	n/a	
Sample size	2076	

Appendix D

Appendix for Chapter 5: Selection on body size: genetic and non-genetic determinants of associations between size and survival

D.1. Additional tables

Table D.1. The percentages of the explained survival variance accounted for by each variance component (random effect) in Models 2 and 3.

Random effect	Model 2	Model 3
nest ID	58.21	57.51
hatch date	22.99	23.69
cohort	5.66	5.35
additive genetic effect	13.14	13.45

There was a slight disagreement between the credible intervals (95% CIs) for the covariances and correlations of hatch date and cohort: the CIs for the correlations did not span zero, implying that these correlations were statistically significant. As in Model 2, these differences were due to the skew in the distributions of the correlations: the shortest interval which contains 95% of the probability was chosen for the calculations and therefore the fat-tailed end of the distribution - containing most of the information - was preferred over the long-tailed end. To obtain a further measure of "significance" for the correlations, I calculated the percentage of negative correlation estimates for these random effects for Models 2 and 3 (Table D.1).

Table D.2. Comparison of credible intervals (95% CIs) for the correlations between mass and survival due to random effects with the percentage of correlation estimates that were negative, and with the 2.5 and 97.5 percentiles. Figures are given for Model 2 (a) and for Model 3 (b).

(a) Model 2

Random effect	**correlation estimate**	**credible intervals (95% CIs)**	**negative correlation estimates (%)**	**percentiles (2.5 and 97.5)**
nest ID	0.06	-0.04, 0.15	11.30	-0.04, 0.16
hatch date	**0.65**	**0.12, 0.99**	**2.80**	**-0.01, 0.96**
cohort	**0.58**	**0.08, 1.00**	**3.60**	**-0.05, 0.95**
additive genetic effect	-0.06	-0.46, 0.32	62.00	-0.47, 0.32
residual variance	0.10	-0.02, 0.21	4.35	-0.01, 0.22

(b) Model 3

Random effect	**correlation estimate**	**credible intervals (95% CIs)**	**negative correlation estimates (%)**	**percentiles (2.5 and 97.5)**
nest ID	0.05	-0.04, 0.15	16.60	-0.05, 0.15
hatch date	**0.70**	**0.23, 1.00**	**1.25**	**0.09, 0.98**
cohort	**0.56**	**0.03, 0.96**	**3.40**	**-0.03, 0.94**
additive genetic effect	-0.09	-0.53, 0.28	67.35	-0.50, 0.32
residual variance	0.11	-0.01, 0.22	3.20	-0.01, 0.22

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